

VALUE OF RAISINS FOR REDUCTION OF OXIDATIVE STRESS, ENDOTHELIAL
DYSFUNCTION, AND INFLAMMATION IN OBESITY

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ABSTRACT

This study investigated the effects of daily consumption of Thompson seedless raisins on markers of inflammation, oxidative stress and endothelial activation in response to an acute high-fat meal in obese individuals. Seventeen overweight men and women consumed raisins or placebo (264 kcal/d) for 14 d in a randomized cross-over design while following a low-flavonoid, weight-maintenance diet. Four high-fat (53% fat) meals were consumed with the respective treatment pre and post interventions. Measures at fasting, and 2, 3 and 4 hours postprandial included markers of oxidative stress (urinary 8-isoPGF_{2α}; serum Oxygen Radical Absorbance Capacity, ORAC), inflammation (serum C-reactive protein, CRP; interleukin-6, IL-6), endothelial function (serum soluble intercellular adhesion molecule-1, sICAM-1; soluble vascular adhesion molecule-1, sVCAM-1), and metabolic measures (free fatty acids (FFA), triacylglycerol (TAC), glucose, insulin). Urinary 8-isoPGF_{2α} decreased 22% and ORAC increased 3% pre to post interventions combined. Postprandial metabolic responses differed by gender, males surpassed females for several measures: FFA, triacylglycerol, glucose, and sVCAM-1. Neither the meals nor treatment with raisins had any noteworthy influence on fasted measures of inflammation or endothelial dysfunction. Acute high fat meal consumption did not result in evidence of inflammation or oxidative stress in these relatively healthy, overweight individuals. Providing all food in regular pattern reduced measures of oxidative stress. Gender influenced metabolic responses to meals; males had a greater postprandial response in metabolic measures than females.

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TABLE OF CONTENTS

Acknowledgements.....	iii
CHAPTER 1: INTRODUCTION.....	1
Introduction.....	2
Statement of the Problem.....	4
Objectives.....	5
Hypothesis.....	5
Delimitations.....	5
Limitations.....	6
Definitions and Symbols.....	7
Basic Assumptions.....	9
CHAPTER 2: REVIEW OF LITERATURE.....	10
Introduction.....	11
Obesity Incidence and Disease Risk.....	12
Obesity and Inflammation.....	13
Obesity, Oxidative Stress, and Inflammation.....	15
Endothelial Dysfunction.....	19
Obesity, Oxidative Stress, Inflammation, Endothelial Dysfunction and Serum Hormones and Metabolites.....	22
Influence of Diet Compliance and Patterns on Oxidative Stress, and Inflammation.....	24
Oxidative Stress, Inflammation, and Endothelial Responses to a Meal Challenge.....	26
Meal Composition Influences on Inflammation, Oxidative stress and Endothelial Dysfunction.....	28
Flavonoids and Raisins.....	30
Summary.....	32

CHAPTER 3: VALUE OF RAISINS FOR REDUCTION OF OXIDATIVE STRESS, ENDOTHELIAL DYSFUNCTION, AND INFLAMMATION IN OBESITY	38
Abstract.....	39
Introduction.....	40
Methods.....	42
Subjects.....	42
Study Design.....	42
Diet Records and Controlled Feeding Menus.....	43
Blood Collection.....	44
Sample Analysis.....	44
Urine Collection.....	45
Raisin Analysis.....	45
Statistical Analysis.....	46
Results.....	47
Subjects.....	47
Diet Records.....	47
Weight Maintenance.....	48
Fasting Blood.....	48
Indices of Initial Health Risk of Subjects.....	48
Postprandial Blood.....	49
Raisin Analysis.....	49
Discussion.....	50
Summary and Conclusions.....	53
References.....	54
 CHAPTER 4: SUMMARY AND RECOMMENDATIONS.....	69
Ideas for Future Research.....	75
 Appendix A: Comprehensive Description of Research Methods and Procedures.....	77
Subject Recruitment and Qualifying Screening.....	78

Comprehensive Baseline: Blood Draws and Anthropometric Measures.....	79
Meal Challenge.....	79
Dispensing of Treatment and Storage: Raisin and Jelly Candy.....	80
Treatment.....	80
Controlled Feedings.....	80
Controlled Feeding Menus	81
Food Procurement.....	81
Washout and Crossover.....	81
24 hour Recall.....	81
Urine Collection and Analysis.....	82
Blood Collection and Analysis.....	82
Raisin Extraction.....	85
Total Phenolic Extraction.....	85
 Appendix B: Individual Values for Lab Measurements.....	 86
 Appendix C: Statistical Analyses of Lab Measurements.....	 112
Statistical Procedures.....	113
 Appendix D: Health History Questionnaire and Supplemental Questions.....	 123
 Appendix E: Infection Questionnaire.....	 128
 Appendix F: Institutional Review Board Request.....	 132
 Appendix G: Informed Consent for Participants of Investigative Projects.....	 144
 Appendix H: Instructions for Subjects.....	 149
Instructions for filling out a Food Record	150
Food Intake Record.....	151
Description of Study.....	152

Low-Flavonoid Diet.....	153
Instructions for Screening Blood Draw.....	155
Instructions for Controlled Feeding	156
Instructions for Treatment Consumption.....	157
Appendix I: Meal Challenge Composition and Controlled Feeding Menu Example.....	158
Meal Challenge Composition.....	159
2500 kcal Day: Sample Menu during Controlled Feeding Period	160
Appendix J: Subject Data Sheets.....	161
Appendix K: Exit Survey.....	165
Appendix L: Recruitment Fliers and email.....	168
References.....	172

List of Tables

Chapter 2 Tables

Table 2.1. Comparison of Serum Markers: Lean and Obese.....	33
Table 2.2. Markers of Oxidative Stress in the Obese.....	34
Table 2.3. Meal Challenges: Composition.....	35
Table 2.4. Metabolic and Endothelial Responses to Meals.....	36
Table 2.5. Raisin and Grape Composition.....	37

Manuscript Tables

Table 3.1. Sample Menu from Controlled Feeding.....	59
Table 3.2. Initial Characteristics of the Study Population.....	60
Table 3.3. Initial Characteristics of the Study Population by Gender.....	61
Table 3.4. Frequency of Elevated Indices of Health Risk.....	62

Table 3.5. Fasting Values for Pre and Post Interventions.....	63
Table 3.6. Postprandial Changes: Raisins and Placebo.....	64

Appendix Tables

Table B1. Raw Data for Descriptives.....	87
Table B2. Raw Data for Subject Weights (kg) (#1-9).....	88
Table B3. Raw Data for Subject Weights (kg) (#10-20).....	89
Table B4. Raw Data for Four Day Food Records (Average).....	90
Table B5. Raw Data for Individual Subject 24 hr Recall.....	91
Table B6. Raw Data for Urinary 8-iso-Prostaglandin F2 α pg/mg Creatinine.....	92
Table B7. Raw Data for Urinary Creatinine mg/dL.....	93
Table B8. Raw Data for Serum Total Oxygen Radical Antioxidant Capacity (ORAC _{total})(TE).....	94
Table B9. Raw Data for Serum Oxygen Radical Antioxidant Capacity (ORAC _{pca}) perchloric acid (PCA)(TE).....	95
Table B10. Raw Data for Serum C-Reactive Protein (CRP) (mg/L).....	96
Table B11. Raw Data for Serum Interleukin-6 (IL-6) (pg/mL).....	97
Table B12. Raw Data for Serum Soluble Intercellular Adhesion Molecule-1 (sICAM-1) (ng/mL).....	98
Table B13. Raw Data for Serum Soluble Vascular Cellular Adhesion Molecule-1 (sVCAM-1) (ng/mL).....	99
Table B14. Raw Data for Serum Free Fatty Acids (FFA) (mEq/L).....	100
Table B15. Raw Data for Serum Triglycerides (TAC) (mg/dL).....	101
Table B16. Raw Data for Serum Glucose (mg/mL).....	102
Table B17. Raw Data for Serum Insulin (uIU/mL).....	103
Table B18. Percent Change C-Reactive Protein (CRP) (%).....	104
Table B19. Percent Change Interleukin-6 (IL-6) (%).....	105
Table B20. Percent Change Soluble Intercellular Adhesion Molecule-1 (sICAM-1) (%).....	106
Table B21. Percent Change Soluble Vascular Cellular Adhesion	

Molecule-1 (sIVAM-1) (%).....	107
Table B22. Percent Change Free Fatty Acids (FFA) (%).....	108
Table B23. Percent Change Triacylglycerol (TAG) (%).....	109
Table B24. Percent Change Glucose (%).....	110
Table B25. Percent Change Insulin (%).....	111
Table C26. RMANOVA Fasting Urinary 8-iso-Prostagandin F2 α	115
Table C27. RMANOVA Fasting Serum Total Oxygen Radical Antioxidant Capacity (ORAC _{total})	115
Table C28. RMANOVA Fasting Serum Oxygen Radical Antioxidant Capacity perchloric acid (ORAC _{pca}).	115
Table C29. RMANOVA Fasting Serum C-Reactive Protein (CRP).....	116
Table C30. RMANOVA Fasting Serum Interleukin-6 (IL-6).....	116
Table C31. RMANOVA Fasting Serum Soluble Intercellular Adhesion Molecule-1 (sICAM-1).....	116
Table C32. RMANOVA Fasting Serum Soluble Vascular Cellular Adhesion Molecule-1 (sVCAM-1).....	117
Table C33. RMANOVA Fasting Serum Free Fatty Acids (FFA).....	117
Table C34. RMANOVA Fasting Serum Triacylglycerol (TA).....	117
Table C35. RMANOVA Fasting Serum Glucose.....	118
Table C36. RMANOVA Fasting Serum Insulin.....	118
Table C37. RMANOVA Percent Change C-Reactive Protein (CRP) (%).....	118
Table C38. RMANOVA Percent Change Interleukin-6 (IL-6) (%).....	119
Table C39. RMANOVA Percent Change Soluble Intercellular Adhesion Molecule-1 (sICAM-1) (%).....	119
Table C40. RMANOVA Percent Change Soluble Vascular Cellular Adhesion Molecule-1 (sVCAM-1) (%).....	119
Table C41. RMANOVA Percent Change Free Fatty Acids (FFA) (%).....	120
Table C42. RMANOVA Percent Change Triacylglycerol (TAG) (%).....	120
Table C43. RMANOVA Percent Change Glucose (%)	121
Table C44. RMANOVA Percent Change Insulin (%).....	121

Table C45. Paired t-test for Initial Subject Characteristics.....	122
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List of Figures

Manuscript Figures

Figure 3.1. Study Design.....	65
Figure 3.2. Interaction of Gender with Time for Postprandial Soluble Vascular Cellular Adhesion Molecule-1	66
Figure 3.3. Interaction between Gender and Time for Postprandial Serum Free Fatty Acids.....	67
Figure 3.4. Interaction between Gender and Time for Postprandial Serum Glucose.....	68

CHAPTER 1:

Introduction

INTRODUCTION:

Overweight and obesity are associated with increased health risk for chronic diseases such as diabetes, cancer, and cardiovascular disease (CVD) (1, 2). Chronically elevated oxidative stress and inflammation, illustrated by increases in oxidatively modified macromolecules (DNA, lipids, carbohydrates, and proteins) (3), serum interleukin-6 (IL-6) (4), C-reactive protein (CRP) (5), and markers of endothelial activation, (serum soluble intercellular adhesion molecule-1, sICAM-1; soluble vascular adhesion molecule-1, sVCAM-1) (5, 6), are related to adiposity and predict higher risk of cardiovascular disease. Elevated metabolic measures (free fatty acids (FFA), triacylglycerol (TAG), glucose, and insulin) have also been shown to have a similar association (4, 7). Studies have demonstrated that high fat meals acutely increase inflammatory or oxidative stress markers, tumor necrosis factor- α (TNF- α), IL-6, CRP, F₂-isoprostanes (8-iso prostaglandin F_{2 α} (8-iso PGF_{2 α})), sICAM-1, and sVCAM-1 (8, 9). The addition of antioxidants vitamins E and C, flavonoids, and adherence to the Mediterranean diet, have been shown to ameliorate these effects, suggesting that this response is linked to the ability of the body to quench free radicals (10, 11). As much of the day is spent in the postprandial state, it is of interest to determine whether addition of foods containing antioxidant compounds to the daily diet of overweight individuals could reduce their basal as well as postprandial inflammatory state, and thus reduce oxidative stress and endothelial dysfunction.

Several studies have shown that high fat meals acutely increase some of the inflammatory or oxidative stress markers. For example, Nappo et al. found that a high-fat meal increased plasma concentrations of inflammatory markers TNF- α , IL-6 CRP, sICAM-1, and sVCAM-1 in type 2 diabetics as well as normal controls when compared to an isocaloric low-fat meal (8). The addition of antioxidants E and C ameliorated these effects more notably in controls, however these findings suggest that this response is linked to the ability of the body to quench free radicals (8). As much of the day is spent in the postprandial state, it is of interest to determine whether addition of nutrients of foods to the daily diet of overweight individuals could reduce their basal as well as postprandial inflammatory state.

A variety of foods contain vitamins or other food components with antioxidant properties. The Mediterranean diet, for example, contains high amounts of fruits, vegetables, and wine. Several studies have reported a reduction in incidence of CVD (12, 13) and serum

CRP (11) in relation to this diet. However, it is unclear what particular component of the diet is most important in reducing inflammation and risk of disease.

Fruits are known to have a variety of polyphenolic constituents such as flavonoids, phenolic acids and tannins (14). In epidemiological studies, such as the Zutphen Elderly Study and the Rotterdam study, investigators demonstrated that individuals in the highest tertile for flavonoid consumption had a 58% reduced risk of CVD compared to individuals with lower flavonoid consumption (15), and that dietary flavonoids from sources such as tea consumed over a number of years was associated with a lowered CVD risk (16). Although there is evidence that a flavonoid-rich diet is associated with reduced health risk, it is still unclear whether or not this is due to a reduction in oxidative stress. Some investigations have explored the effects of total fruit and vegetable intake on markers of oxidative stress. Kim et al. (14) showed an increase (41%) in erythrocyte superoxide dismutase (SOD) enzyme activity in normal weight, non-smoking young females after subjects consumed a phenol-depleted diet for 6 days, thus indicating the compensation of endogenous antioxidant systems in the absence of adequate dietary antioxidants. By the same token, Cao et al. (17) found significant positive correlations between fasting oxygen radical scavenging capacity (ORAC) of plasma in healthy nonsmokers and the daily consumption of 10-12 servings of fruits and vegetables. Modification of one high flavonoid food, Concord grape juice, increased lag-time in LDL susceptibility to oxidation after daily consumption (18). Similar improvement in oxidative state were seen in an investigation by Zern et al. (19) of the effects of a daily consumption of 36g of a lyophilized grape powder supplement (equivalent to 1.5 cups of fresh grapes) to reduce TNF- α as well as urinary measures which were significantly ($p < 0.05$) reduced by the intervention.

Increases in oxidative stress and chronic inflammation are also associated with poor endothelial function (20), impaired vasodilation being the key step in CVD progression. However, the link between dietary components, oxidative state and endothelial function remains unclear. It is believed that one of the mechanisms involves the disruption of nitric oxide bioavailability (10, 21). Several investigations have looked at the effects of flavonoid rich foods and oxidative mechanisms as well as vasodilatory capabilities. For example, Vogel et al. (22) saw a 71% improvement in flow mediated vasodilation (FMD) when vitamin E and C were added to a high fat meal (50% fat) of olive oil and bread (22). This suggests an oxidative mechanism involved in the disruption of endothelial function. Activated endothelial cells express adhesion

molecules aiding in the recruitment and infiltration of monocytes (23), another key step in disease progression. This association was investigated by Blanco-Colio et al. by supplementing a high fat breakfast meal with red wine (24). The addition of an antioxidant rich beverage with the high fat meal reduced the expression of the redox sensitive nuclear transcription factor κ B (NF- κ B) involved in the transcription of inflammatory molecules including adhesion molecules (24).

There is a good amount of data supporting the health benefits of flavonoids (13, 25, 26). Wines, grapes, and raisins are high in flavonoids such as resveratrol (wine and grapes only), quercetin, kaempferol, (+)-catechin, and (+)-epicatechin (18, 27, 28). Grapes, grape juice, and grape seed extract have been shown in clinical studies to have similar effects to that of wine on the preservation of NO and the decrease in endothelial activation (29, 30). Following this logic, raisins may also exhibit similar health benefits. Seedless raisins are one of the Key Foods identified by the United States Department of Agriculture (USDA) (31), suggesting they are a significant source of nutrients pertinent to health concerns in the US population. Among other important nutrients, raisins contain 359.5 mg total phenols per 90g serving compared to 142.2 mg for green and 198.5 mg for red grapes (also 90 gram servings) (32).

To date, no investigations utilizing Thompson seedless raisins as a dietary intervention to reduce inflammation have been reported. It may be of interest to investigate the effects of acute and chronic consumption of seedless raisins on markers of oxidative stress, inflammation, and endothelial activation in the fasted state, and in response to an acute high-fat meal challenge in overweight and obese subjects. Pursuing this line of investigation may help determine whether a dietary intervention utilizing a whole food source of phenolic compounds would be capable of disconnecting obesity from some of the associated health problems.

STATEMENT OF THE PROBLEM:

Obese and overweight individuals are already at risk of developing CVD based on their increased adiposity alone. Higher BMI and hip to waist ratios are associated with elevated risk of developing CVD, diabetes, cancers and other chronic diseases. Additionally, these same measures of adiposity have also been associated with markers of inflammation and oxidative stress which have been seen in these populations. It is known that interventions involving the administration of antioxidants can help reduce these same markers, thus implying that the intake

of antioxidants can ameliorate the effects of increased adiposity. The typical diet that lends to increased adiposity is one of high energy, with the greatest proportion of calories consumed in the form of fat. Additional investigations implicate that the consumption of a single high calorie/high fat meal can act as a metabolic stressor. This effect is more pronounced in populations with an elevated baseline of these inflammatory and metabolic markers, such as the overweight and obese. It can be assumed that the average individual may eat anywhere from 1 to 3 meals per day, thus spending a great amount of there time in a postprandial state. Thus far the research exploring the effects of a single whole food as an antioxidant and anti-inflammatory has been limited. Research has often used broad classes of foods, such as the Mediterranean style diet, to support their arguments. Further investigation is warranted in this area, especially in the area of easily procured, versatile and affordable foods, such as raisins.

OBJECTIVES:

- To determine if raisins have the ability to ameliorate the increased inflammation and oxidative stress while fasted or induced by a high fat meal in overweight individuals.
- To investigate the link between oxidative stress, inflammation, and endothelial function in overweight/obese populations.

HYPOTHESES:

H₀: Regular consumption of 90 grams of raisins will not reduce the oxidative stress, endothelial dysfunction, and inflammation in the fasted state or in response to a high fat meal challenge in overweight individuals compared to an isoenergetic carbohydrate snack.

H₀: A high-fat meal challenge of >700 kcal, and >48% fat will not induce oxidative stress, inflammation, and endothelial dysfunction in overweight individuals.

H₀: There will be no significant link found among oxidative stress, inflammatory, or endothelial markers in the fasting or in response to a high fat meal challenge in overweight or obese individuals.

DELIMITATIONS:

- The subjects were screened to insure:
 - The subjects were overweight or obese males or females with a BMI > 27 and between the ages of 18 and 50 years old.
 - Subjects with abdominal obesity (waist circumference > 102 cm for men and >88 cm for women) determined by measurement with a tape measure.

- No known diagnosed CVD, diabetes, hypertension, or inflammatory conditions such as Lupus or Inflammatory Bowel Disease (IBD).
- No tobacco use.
- No unstable weight (± 0.5 kg) with in the past 3 months.
- Subjects were to refrain from strenuous exercise (> 120 min/wk of moderate to intense activity).
- No use of medications known to influence inflammation.
- Subjects could not follow a vegetarian or vegan diet due to controlled feeding period menu limitations.
- Subjects were randomly placed in treatment groups.
- Subjects were to remain on a low-flavonoid diet for the duration of the study: Two weeks prior to the first intervention and through the end of the last treatment, for a total of 8 weeks.
- Baseline fasting serum and urine was collected two weeks prior to the first intervention.
- Blood was collected on each of the four meal challenge test days at baseline (12 hr, overnight fast), 120, 180, and 240 minutes after the meal challenge.
- Menus were adjusted to accommodate multiple calorie levels; 1700, 2000, 2500, 3000, and 3500 kcal/day for weight stability.
- Two random 24 hr recalls (phone): During the self-selected diet period and wash-out period. 4-day food records were collected prior to the initiation of the study.
- The independent variable for treatment: Raisin treatment was two 45 gram servings (132 kcal) with the morning and evening meals for two weeks. Placebo treatment was two 35 grams servings (132 kcal) of jelly beans with the evening and morning meals for two weeks.
- The independent variable for meal challenge was a slightly modified McDonalds Big Breakfast®. Providing 900 kcal (53% fat, 34% CHO, 14% PRO).
- Dependant variables were oxidative stress marker: urinary isoprostanes (8-epi $\text{PGF}_{2\alpha}$); Inflammatory markers, serum CRP and IL-6; Endothelial function, sICAM-1 and sVCAM-1; Hormones and Metabolites, insulin, TAC, FFA and glucose.

LIMITATIONS:

- Subjects were free living. Therefore self reported diet records, low-flavonoid diet compliance, and treatment compliance could not be confirmed.
- Controlled feeding periods did not reflect habitual eating patterns of the subjects.
- Results of the investigation can only be applied to a population ages 18-50 with a BMI >27 .
- Results cannot be applied to subjects with disease, including diabetes, as subject selection excluded these individuals.
- Changes in exercise habits were not formally assessed over the course of the 8 week investigation.

DEFINITIONS AND SYMBOLS:

- 8-epi PGF_{2α} 8-epi-prostaglandin F_{2α}: is produced *in vivo* by free radical-dependent peroxidation of lipid-esterified arachidonic acid.
- CRP C-Reactive Protein: Acute phase serum protein of Inflammation secreted by hepatocytes.
- IL-6 Interleukin-6: Pro-inflammatory cytokine. One role includes the induction of the hepatic production of CRP.
- sICAM-1 Soluble Intercellular Adhesion Molecule-1: A type of cellular adhesion molecule expressed in the endothelium involved in the tethering of leukocytes (white blood cells).
- sVCAM-1 Soluble Vascular Adhesion Molecule-1: A type of cellular adhesion molecule expressed in the endothelium involved in the tethering of leukocytes (white blood cells).
- TAG Triacylglycerol
- FFA Free Fatty Acid
- CHO Carbohydrate
- PRO Protein
- CVD/CHD Cardiovascular Disease/Coronary Artery Disease: Include many diseases that affect the heart organ or associated vascular system. The primary conditions are arteriosclerosis, coronary artery disease, and peripheral artery disease.
- DNA Deoxyribonucleic acid: Genetic material (nucleic acid).
- NF-κB Nuclear factor kappa B: Redox sensitive nuclear transcription factor involved in immune responses (inflammation).
- TNF-α Tumor necrosis factor-α: Inflammatory cytokine involved in the mediation of other inflammatory cytokines (example: IL-6).
- Flavonoid A class of polyphenolic compounds which function as biological antioxidant. Prevalent in fruits and vegetables.
- HTN Hypertension: Defined as blood pressure >140/90 mmHg.
- Fibrinogen Blood protein involved in clotting.
- PAI-1 Plasminogen activator inhibitor-1: Blood protein involved in the inhibition of anti-clotting factors.
- SAA Serum amyloid A: Acute phase serum protein of Inflammation secreted by hepatocytes.

• LDL	Low density lipoprotein: Produced in the liver.
• Leptin	A hormone (protein) that regulates energy intake and expenditure.
• BMI	Body mass index: An estimated of obesity utilizing height and weight: Weight in kilograms (kg) divided by height in meters (m) squared.
• WHR	Waist to hip ratio: A measure of abdominal obesity: waist circumference > 102 cm for men and >88 cm for women).
• Type 2 diabetes	A disorder involving abnormal blood glucose metabolism.
• HbA _{1C}	Hemoglobin A1C: Glycosylated hemoglobin (non-enzymatic).
• ROS	Reactive oxygen species: Unstable, reactive molecules with an unpaired electron; free radical.
• UCP	Uncoupling Protein: Located in the mitochondrial inner membrane. These channel protein allow electrons to bypass the formation of ATP (adenosine triphosphate).
• ORAC	Oxygen radical absorbance capacity: A quantitative method used for assessing the overall radical scavenging potential of a biological sample.
• ORAC _{total}	Oxygen radical absorbance capacity: Of all the sample constituents.
• ORAC _{pca}	Oxygen radical absorbance capacity: The protein-free portion of a sample.
• ELISA	Enzyme-Linked Immunosorbent Assay: A method used for assaying protein concentrations of biological samples.
• eNOS	Enzyme that catalyzes the formation of nitric oxide (NO). NO is a compound that induces vasodilation in the endothelium.
• NADPH	Nicotinamide adenine dinucleotide phosphate: Energy substrate involved in hydrogen transfer.
• FMV/FMD	Flow-mediated vasodilation measured in the brachial artery. Used to evaluate endothelial function.
• PMN/MNC	Polymorphonuclear leukocytes/mononuclear cells: White blood cells that produce ROS as a function of the immune system.
• OGTT	Oral glucose tolerance test: Diagnostic tool used for Type 2 Diabetes.
• (RM) ANOVA	(Repeated Measures) Analysis of Variance

BASIC ASSUMPTIONS:

- Subjects were 100% compliant with the low-flavonoid diet for the duration of the 8 week intervention.
- Subjects were 100% compliant with their assigned treatment.
- Flavonoid content was 100% absorbed.
- Subjects were 12h fasted prior to all four meal challenges as well as initial baseline measures prior to the initiation of the study.
- All diet records and recalls were 100% accurate.
- All health histories were 100% accurate.
- Subjects abstained from all anti-inflammatory medications throughout the 8 weeks of the study, as well as the 2 weeks prior to the start of the study.

CHAPTER 2:
Review of the Literature

INTRODUCTION:

Overweight and obesity are associated with increased health risk for chronic diseases such as diabetes, cancer, and cardiovascular disease (CVD) (1, 2). Chronically elevated oxidative stress and inflammation, illustrated by increases in oxidatively modified macromolecules (DNA, lipids, carbohydrates, and proteins) (3), serum interleukin-6 (IL-6) (4), C-reactive protein (CRP) (5), and markers of endothelial activation, (serum soluble intercellular adhesion molecule-1, sICAM-1; soluble vascular adhesion molecule-1, sVCAM-1) (5, 6), are related to adiposity and predict higher risk of cardiovascular disease. Elevated metabolic measures (free fatty acids (FFA), triacylglycerol (TAG), glucose, and insulin) have also been shown to have a similar association (4, 7). Studies have demonstrated that high fat meals acutely increase inflammatory or oxidative stress markers, tumor necrosis factor- α (TNF- α), IL-6, CRP, F₂-isoprostanes (8-iso prostaglandin F_{2 α} (8-iso PGF_{2 α})), sICAM-1, and sVCAM-1 (8, 9). The addition of antioxidants vitamins E and C, flavonoids, and adherence to the Mediterranean diet have been shown to ameliorate these effects, suggesting that this response is linked to the ability of the body to quench free radicals (10, 11). As much of the day is spent in the postprandial state, it is of interest to determine whether addition of foods containing antioxidant compounds to the daily diet of overweight individuals could reduce their basal as well as postprandial inflammatory state, and thus reduce oxidative stress and endothelial dysfunction.

Fruits are known to have a variety of polyphenolic constituents such as flavonoids, phenolic acids and tannins (14). Epidemiological studies looking at overall vegetable, fruit, wine and other grape product consumption demonstrate an association with lower CVD (13, 15), suggesting that flavonoids or other compounds found in these foods are responsible for the effect on disease risk. Although there is evidence that a flavonoid-rich diet is associated with reduced health risk and CVD (13), it is still unclear whether or not this is due to a reduction in oxidative stress. Some investigations have explored the effects of total fruit and vegetable intake on markers of oxidative stress, showing the reduction in several markers (14, 18, 19). Several investigations have found positive outcomes when looking at the effects of flavonoid rich foods, oxidative mechanisms, as well as vasodilatory capabilities (22-24). Wines, grapes, and raisins are whole foods that are high in flavonoids (27, 28). Studies showing health benefits of wine and grape juice suggest that raisins may have similar benefits as their non-dried counterparts (13, 33,

34). To date, no investigations utilizing seedless raisins as a dietary intervention to reduce inflammation have been done and further investigations are warranted. The remainder of this review will summarize the scientific literature related to this topic.

OBESITY INCIDENCE AND DISEASE RISK

The incidence of overweight and obese individuals in the USA is increasing. Data from the National Health and Nutrition Survey (NHANES III) show that the prevalence of overweight and obese individuals in the US exceeds 64% and 30%, respectively (2). Obesity itself is a risk factor for multiple chronic diseases and conditions associated with oxidative stress. The scope of potential diseases or conditions includes; arthritis, hypertension (HTN), dyslipidemia, diabetes, stroke, cardiovascular disease (CVD), polycystic ovary disease, sleep apnea and cancer (2, 35). It is well known that interventions that encourage changes in lifestyle can reduce the risk of developing these conditions (1).

Of the chronic diseases associated with the overweight and obese populations, Type 2 Diabetes is one of the most widespread (2). In 2007 the prevalence of total diabetes (diagnosed and undiagnosed) in the US was 23.6 million people representing 7.8% of the total US population (2). Individuals with diabetes are at higher risk of developing complications such as heart disease, which is the leading cause of diabetes related deaths in the US (2). Adults with diabetes have a 2-4 times higher rate of coronary heart disease (CHD) related deaths than individuals without diabetes, and approximately 65% of deaths in the diabetic population are due to heart disease or stroke (2). A state of increased oxidative stress and chronic inflammation is believed to be the link between all of these conditions (36, 37).

The health risks of obesity, particularly visceral adiposity, is independently associated with elevated CVD risk (38). In 2002 the metabolic syndrome (pre-diabetes), was estimated to affect 47 million people in the US population (2). According to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) definition, an estimated 24% of the US population is afflicted with this metabolic disorder (39). This disorder or clustering of metabolic abnormalities is defined by the diagnoses of at least three of the following criteria: Abdominal (visceral) obesity (defined by waist circumference), insulin resistance or impaired glucose tolerance, HTN, dyslipidemia, pro-thrombotic (elevated blood fibrinogen or

plasminogen activator inhibitor–1(PAI-1)), and pro-inflammatory state (elevated blood CRP). Individuals diagnosed with the metabolic syndrome are at high risk of developing diabetes and CVD (2, 39).

As obesity is on the rise, as well as the prevalence of diagnosed Type 2 diabetes, and that these conditions raise CVD risk, it is relevant that investigations are aimed at finding the common denominator(s) as well as interventions to reduce risk.

OBESITY AND INFLAMMATION

Inflammation is a response of the innate immune system to various stimuli (40). This defense system is coordinated by a complex group of reactions which is initiated when phagocytosis alone fails to control infection or when there is tissue injury (41). Inflammation is composed of four different components; activation of clotting mechanisms, increased blood flow, increased capillary permeability, and a flood of phagocytic cells (neutrophils, macrophages, and lymphocytes) (41). If the inflammatory response is prolonged, acquired immunity responses take effect including antibodies and molecules from activated lymphocytes which attach to up-regulated cell adhesion molecules in the endothelium (23).

There are many factors that help to instigate and maintain a state of inflammation. Many of these processes are regulated by chemical messengers that can modify particular immune responses in the body. Important biologically active compounds that have been identified as key participants in this process include, TNF- α , IL-6, and CRP. These particular cytokines have been identified as risk factors for CVD and have been found to be elevated in obese populations (42).

TNF- α is one of the chief cytokines that aids in mediation of immune and inflammation processes (43). This cytokine induces PAI-I (promotes clotting) secretion from the adipose in order to control areas of infection (38). Jointly this vital role in infection control also increases the likelihood of thrombotic events when at elevated levels in the obese (44). Elevated CRP levels have been associated with an increased CVD risk because atherosclerosis is known to persist as an inflammatory state in the vasculature (44).

IL-6 is a key regulator of the immune systems acute phase protein synthesis in the liver (44), and is produced by macrophages in response to trauma, sepsis, and other inflammatory conditions (44). This response stimulates the secretion of soluble proteins such as CRP, serum

amyloid A (SAA), and fibrinogen (45). These proteins represent just three of the 45 different acute phase proteins associated with the inflammatory response (45). In response to IL-6 production, CRP usually rises quickly and then falls as the inflammatory response subsides (45). CRP binds to receptors in order to induce the production of pro- and anti-inflammatory cytokines, as well as to aid in the phagocytosis of bacteria (45). CRP located in vessel walls help induce the uptake of LDL via macrophages, as well as the up-regulation of endothelial adhesion molecules (46), a key step in atherosclerosis. These inflammatory responses are present in obese individuals, the defining difference being that the response persists at a low level without any known acute event and does not subside without intervention (weight loss, pharmaceutical, etc.) (47).

Adiposity influences levels of serum cytokines. It has been estimated that approximately 30% of total circulating concentrations of IL-6 are produced by the adipose tissue in healthy subjects (48). Logically, an increase in adiposity could be related to an increase in circulating inflammatory markers (Table 1). Macchi et al. (49) demonstrated that among a group of non-smoking, non-diabetic obese women, there was a significant correlation between CRP and circulating adipokines (IL-6, leptin, TNF- α , plasminogen, and PAI-1. This relationship remained even after being adjusted for fat mass (49). Other investigators have established similar relationships as Maachi's group. Giugliano et al. (50) and Brook et al. (51) have reported that elevated levels of pro-inflammatory cytokines, IL-6, IL-18, TNF- α , and CRP are associated with high body fat and Body Mass Index (BMI) and an increase in CVD risk factors. TNF- α has been shown to decrease with weight loss thus supporting a correlation between elevated levels of this cytokine and BMI (43, 52). Furthermore, because TNF- α can originate from adipocytes its secretion has been associated with a BMI-related increase in insulin resistance (43).

Other than adipose tissue, the major sources of pro-inflammatory cytokines include the endothelium and white blood cells (53). Circulating IL-6 and CRP may originate from activated leukocytes, and to a lesser degree from fibroblasts and endothelial cells (46, 53). This has important implications in the progression of atherosclerosis, because of the enhanced infiltration of white blood cells through the endothelium perpetuates the inflammatory process in the vasculature (46).

In obese and chronic disease populations, the most common quantitative markers for assessment of inflammation include: plasma or serum concentrations of CRP, IL-6, TNF- α ,

plasminogen, PAI-1, and leukocyte counts (38, 54). The major limitations encountered when evaluating markers of inflammation are the many confounding variables that could give misleading values. High values may be misinterpreted if an individual presents with an infection or an injury that may have an inflammatory response. Conversely, anti-inflammatory medications can give depressed values for inflammatory markers. It is crucial to control for such variables such as illness and injury, or pharmaceutical interventions that would generate erroneous data.

Adipose distribution plays another role in promoting the inflammatory state (49). Visceral obesity produces proportionately larger concentrations of TNF- α and IL-6, known inflammatory response mediators (55). Assessment of visceral obesity can be easily estimated by simple waist circumference or waist to hip ratio measurements (56). This type of tool has been used as reliable clinical tools of assessment (56). It is essential that not only an estimate of general adiposity, such as a BMI calculation, be used to identify individuals at risk for CVD. Measures of visceral or central adiposity have an even stronger relationship with CVD risk and may prove to be a more valuable assessment tool.

OBESITY, OXIDATIVE STRESS, AND INFLAMMATION

Oxidative stress has been suggested to play a significant role in the pathogenesis of various chronic diseases (18, 57). Populations that have been identified as having elevated oxidative stress include obese individuals, those with CVD, cancer, diabetes, and other chronic diseases (57). It has been demonstrated through a variety of known biological oxidative measures that obesity alone can contribute to oxidative stress (Tables 1, 2), and that this population is at risk even before any diagnosis of chronic disease.

Oxidative stress results from endogenous generation or external high exposure to oxidants that result in free radicals and reactive oxygen species ROS, such as super oxide. Free radicals are introduced to biological systems either by endogenous origins such as by-products of cellular metabolism (oxygen metabolism, respiratory burst, apoptosis and coagulation), or exogenous sources (UV light, exercise, diet, cigarette smoke, and ozone) (3). As stated before, free radicals/oxidants can overwhelm antioxidant defenses, both enzymatic and/or non-enzymatic, and they can negatively impact macromolecules such as DNA, proteins,

carbohydrates, and lipids, thus resulting in modified or loss of function of these structures. It has been suggested that the modification in the function of these macromolecules is at the root of the pathophysiological changes associated with chronic diseases as well as the general aging process (3). The viscous cycle of oxidative stress and disease progression seen in these conditions can be related to both an existing redox imbalance as well as oxidative processes perpetuated by the disease itself (3). There are various quantitative measures used to evaluate oxidative stress. Which measure utilized in research depends on many factors, some of which include; population of interest, physiological compartment of interest (location of ROS generation), and the mode of intervention (if any).

One of the proposed origins of ROS and oxidative stress in obesity involves the mitochondria. Mitochondrial contributions to superoxide formation through oxygen metabolism add to free radical production (36). Obese individuals have been shown to have a 38% increased consumption of oxygen for the same activity compared to lean individuals (36). The obese may be less energy efficient when performing the same activity and therefore have increased energy expenditure and cellular respiration resulting in an increased flow of electrons through the electron transport chain (ETC). Furthermore, increased cellular respiration is associated with elevated levels of oxidized substrates in the obese (36). Interestingly, mitochondrial uncoupling proteins (UCPs), proteins that allow electrons to leak across the innermembrane and bypass ATP synthesis, are up-regulated in the obese (58). This may occur in response to elevated ROS production resulting from excess energy metabolism, and/or hyperglycemia, insulinemia, and elevated FFA, which are associated with increased ROS as well (40). This suggests that general activities such as walking (36), and hypercaloric diets (40) may participate to increase ROS generation at the mitochondrial level in the obese. If this increased ROS production is not balanced by quenching via antioxidants, oxidative stress and molecular damage can result. Importantly, this could be associated with increased postprandial oxidative stress resulting from the ingestion of a high energy meal.

A few measures of oxidized macromolecules include: DNA oxidation, 8-OHdG (8-hydroxy-2'-deoxyguanosine), measurement of autoantibodies to HMdU (5-hydroxymethyl-2'-deoxyuridine), and the Comet Assay (single cell gel electrophoresis) (59). Measures of protein oxidation include protein carbonyl (*ex vivo*) (60). Measures of carbohydrate oxidation include, advanced glycation end products (AGEs) such as glycated hemoglobin (HbA_{1c}) (59). Finally,

measures of lipid peroxidation include, thiobarbituric acid-reactive substances (TBARS), breath hydrocarbons (59), LDL resistance to oxidation (*ex vivo*), lipid peroxides, and F₂ isoprostanes, such as 8-isoPGF_{2α} (59, 61). In obese populations, the preferred measure is urinary 8-isoPGF_{2α}, which measures whole body oxidative stress (62). Increased urinary excretion of this metabolite has been correlated with increased adiposity in humans (62).

Oxidative stress can also be indirectly measured by evaluating changes in overall antioxidant status (of a biological sample), and is based on the premise that a decrease in antioxidant capacity of a sample can be attributed to oxidative processes. Oxygen radical absorbance capacity (ORAC) is a measure of the total antioxidant capacity of a sample (biological or plant origin). In human serum or plasma, ORAC can be seen as the overall ability of the sample to scavenge oxygen radicals (63). A decrease in ORAC after an oxidative stress challenge can be attributed to the consumption of antioxidants in the sample by free radicals generated as a result of the stressor (63). There are various assays that function under the same principles as ORAC. These assays include; total peroxyl radical trapping (TRAP), trolox equivalent antioxidant capacity (TEAC), ferric reducing ability of plasma (FRAP), ferrous xynol orange (FOX), etc. ORAC may be a superior method due to its unique ability to not only take a reactive species reaction to completion, but to use the area under the curve (AUC) as a measure of quantitation as well (63). This allows the combination of both inhibition time (substrate oxidation) and inhibition percentage of the reactive species into one value (63). This is important because it produces a value that is representative of the total antioxidant capacity of a sample in reference to the oxidative potential of an appropriate biological oxidant (peroxyl radical).

The shortcomings of any oxidative stress measure are usually associated with the specificity of the assay (confounding variables), and the difficulty in determining the actual point of origin. Many of these markers using serum or plasma can be used to demonstrate that oxidative damage has occurred, but not necessarily by which particular mechanism or in which biological compartment. Therefore it can be said that the conclusions regarding where the oxidation has occurred are limited to speculation, and as a result, warrant further investigation and more developed methodologies.

As stated before, of the most appropriate measures for assessment of oxidative damage in the obese are F₂-isoprostanes (62). This particular biomarker is indicative of non-enzymatic *in*

vivo lipid peroxidation resulting from free radical oxidative damage to arachidonic acid, which is predominantly found esterified in the phospholipid bilayer of cellular membranes. Investigators have found elevated levels of plasma or urinary F₂ isoprostanes, typically the metabolite 8-iso prostaglandin F_{2α} (8-iso PGF_{2α}) in the urine in obese populations (Tables 1, 2). The connection between inflammation and oxidative stress with this measure can be connected to the visceral adipose tissue (38). Because this tissue has the ability to secrete inflammatory cytokines such as TNF-α and IL-6 (43), ROS produced in response to this inflammatory condition engage in lipid peroxidation reactions producing free 8-iso PGF_{2α} (64). Additionally, 8-iso PGF_{2α} demonstrates bioactive properties which may contribute to enhanced platelet activation in individuals with known chronic disease (64). It is believed that the increase in lipid peroxidation is related to the extent of adiposity, which is also related to increased secretion of inflammatory markers from the adipose tissue (38). Investigations have shown that weight loss reduces concentrations of 8-iso PGF_{2α} and markers of inflammation in the obese (38, 52), further supporting the relationship between obesity, increased oxidative stress and lipid peroxidation. Davi et al. (38) found that CRP was decreased in women with visceral adiposity after weight loss. Additionally, they saw a relationship between CRP, WHR above 0.86, and the predicted rate of urinary 8-iso PGF_{2α} excretion. Other investigators found similar findings. Bougoulia et al. found significant decreases in serum, IL-6, CRP and 8-iso PGF_{2α} in obese women after a 6 month isocaloric, weight loss intervention (65).

Bougoulia's group collected serum isoprostanes and used an enzyme-linked immunosorbent assay (ELISA) method for establishing concentrations (65). The preferred method for assessment of 8-iso PGF_{2α} is through the use of gas chromatography negative ion chemical ionization mass spectrometry. This method is expensive, time consuming, and not easily accessible for all labs (52). A more affordable but a less certain method of assessment is through ELISA (66). Fortunately, normal ranges for F₂-isoprostanes, have been established in a variety of biological tissues, thus allowing for detection of small deviations and the measurement of slight changes in oxidative stress (62). Investigators have also found that measures of this biomarker are unaffected by ingestion of dietary isoprostanes which may be consumed in the average diet containing fat (62). Consequently the use of urinary 8-iso PGF_{2α} has been demonstrated to be an appropriate measure of oxidative stress in obese populations (62, 64).

ENDOTHELIAL DYSFUNCTION

A constant state of oxidative stress and related inflammation can be very detrimental to vascular function. Atherosclerotic plaques form on sites of stress or injury within the vasculature. It is believed that this process is contributed to by a loss of NO-dependant vasodilation (46). eNOS is an enzyme which functions to produce nitric oxide (NO), a primary constituent involved in vasodilation (46). Interruptions in concentrations of NO, either through a decreased ability to produce, or an increased consumption of NO through oxidative reactions, results in a decreased vasodilatory response. When this occurs, the endothelium loses the ability to respond to mechanical stressors such as increase in blood pressure (BP) putting the cells at risk of injury. Injury to the endothelium helps set the stage for an amplified inflammatory response above that of other oxidative processes, including cell membrane damage through lipid peroxidation as well as modification of other macromolecules (44). These processes only aid in the propagation of disease and CVD risk.

The health of the endothelium depends on many factors. The basic anatomy of the vascular endothelium consists of four layers of tissue (45). Moving from the outside in; the outer most layer is the tunica externa, followed by the tunica media, and then the tunica interna, which is composed of an internal elastic layer and the endothelium (45). Atherosclerosis is characterized by injury done to the endothelium and the formation of fatty deposits in the tunica media (45, 46).

Healthy vascular endothelium not only operates as a modifier of vasomotor function and vascular tone, it plays an essential role in the regulation of inflammation, activation of platelets, leukocyte adhesion, thrombosis, smooth muscle cell proliferation and migration, hyperplasia, and vasospasm (38, 46). The release of nitric oxide (NO) by the endothelium causes relaxation in the vascular smooth muscle cells (67). NO is produced by the enzyme nitric oxide synthase (NOS isoform NOSIII or eNOS) which converts L-arginine to L-citrulline. eNOS activation is induced by a variety of factors such as neurotransmitter acetylcholine (ACh), serotonin, thrombin, bradykinin, and shear stress. In addition to external stimuli, NO production requires the cofactors calmodulin, NADPH, and tetrahydrobiopterin (BH₄). Once NO is produced, it diffuses through the endothelial cell membrane into the smooth muscle cell layer. NO induces

vasorelaxation via the enzyme guanylate cyclase (GC), which converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP) (67).

eNOS uncoupling occurs at the protein strand that connects the enzyme together (Figure 5). When uncoupling happens, electrons donated by NADPH are transferred to molecular oxygen (O_2) rather than L-arginine's amine group, thus producing super oxide (O_2^-) rather than NO. Therefore, eNOS uncoupling not only produces ROS, but reduces NO generation and potential for vasorelaxation (67).

eNOS uncoupling has been implicated as a contributor to endothelial dysfunction in conditions such as hypercholesterolemia, nitrate tolerance, diabetes, and hypertension (67). There are three known mechanisms for eNOS uncoupling: A decreased availability of L-arginine and other cofactors; increased oxidation of BH_4 ; and post-translational protein modification (67). Theoretically, if more ROS are produced via eNOS uncoupling, the modification of proteins through oxidative mechanisms are amplified. Therefore it can be seen as a self-perpetuating cycle of increased oxidative stress and eNOS uncoupling followed by further increased levels of ROS production.

Inflammation exacerbates endothelial dysfunction and can result in plaque instability and rupture leading to thrombosis and cardiovascular events (23, 46). These plaques are unstable due to macrophage enzymatic activities within the fibrous cap, a site often responsible in plaque rupture (46). Thrombus formation is decreased by the inhibition of platelet aggregation, coagulation, and the initiation of fibrinolysis (46). These critical factors in thrombus formation are mediated by proper NO bioavailability in healthy endothelium.

Adhesion molecules are expressed in the endothelium mediated by various factors. ICAM-1 (expressed at basal level) and VCAM-1 (transcriptionally induced), as well as E-selectin are expressed on the surface of endothelium cells and are also found in free soluble forms (23). E-selectin is involved in rolling and tethering of circulating leukocytes, where ICAM-1 and VCAM-1 are involved in firm adhesion of the cells to the endothelium (23). These molecules are increased in response to a variety of stimuli including, elevated inflammatory cytokines ($TNF-\alpha$) (23, 68), hyperglycemia, and hypertriglyceridemia (either postprandial or as a result of a metabolic disorder) (69). It has been proposed that these molecules are increased in response to oxidative stress, and that a possible mechanism could involve NADPH oxidase ROS generation (69). Soluble forms of ICAM-1 (sICAM-1) and VCAM-1 (sVCAM-1) are found in

the plasma due to shedding or proteolytic cleavage (67). In general, adhesion molecules orchestrate the rolling, arresting (firm adhesion), and transmigration (infiltration) of leukocytes into the endothelium (23). This process represents a key step in atherosclerotic plaque formation in the vasculature.

Non-invasive clinical assessments of endothelial function include procedures such as forearm blood flow (FBF) a measure of the response to Ach (vasodilator) by strain gauge plethysmography, and flow-mediated vasodilation (FMV or FMD) measured in the brachial artery (70). Other measures include serum concentrations of sICAM, sVCAM, and E-selectin. Elevated levels of adhesion molecules are indicative of endothelial activation, a key step in the recruitment and infiltration of circulating leukocytes into the endothelium. Both vasodilation and serum measures test the endothelium-dependant vasodilation response as an indirect determination of NO bioavailability. Additional methods for measuring endothelial function include L-arginine and nitroglycerine administration (67).

The use of FMV has been established as a valid measure of endothelial dysfunction and cardiovascular disease risk (71). Because endothelial dysfunction occurs systemically, both in the coronary and peripheral tissues, the use of a brachial artery dilation response to shear stress is an appropriate measure. FMV has been used as a measure of oxidative stress response to meal challenges (72), where a decrease in arterial dilation percentage is indicative of a decrease in NO bioavailability. It is assumed that the NO is either consumed in oxidative reactions or the generation of NO is inhibited through some other oxidative mechanism. Evidence to support an oxidative interference is implied when FMV is partially restored by antioxidant supplementation (22). Soluble adhesion molecules have been shown to correlate well with FMV measures (73). Lupattelli et al. found a significant inverse relationship between FMV and sICAM-1 and sVCAM-1 in fasting subjects with hypertriglyceridemia compared to normal healthy controls (73). Due to adhesion molecules roll in leukocyte infiltration into the endothelium, an increase in the soluble form (in serum) of these molecules can be used as markers of endothelial dysfunction.

Overweight and obese populations have been found to have increased serum concentrations of sVCAM and sICAM (Table 1), indicating a relationship between this population and endothelial activation (51). Because this population has elevated inflammatory markers, and that under these conditions NO bioavailability is compromised, these individuals

are at an increased risk for CVD. Luc et al. (6), using data collected from the PRIME study, compared plasma concentrations of sICAM-1 and sVCAM-1 of 318 males with coronary heart disease (CHD) and 613 healthy controls. Plasma concentrations of soluble adhesion molecules were found to have a significantly positive correlation with BMI, TG, and CRP levels, with sVCAM-1 being slightly lower. Furthermore, it was shown that elevated levels of sICAM-1 were significantly associated with an increased risk of CVD (6). These findings suggest that increased concentrations of soluble adhesion molecules are associated with an increase in BMI and an increased risk of CVD.

OBESITY, OXIDATIVE STRESS, INFLAMMATION, ENDOTHELIAL DYSFUNCTION AND SERUM HORMONES AND METABOLITES

Obese populations and individuals with the metabolic syndrome or diabetes are at higher risk for CVD due to other factors such as elevated concentrations of free fatty acids, blood glucose, (Table 1, 4) and insulin resistance (8, 50). Insulin resistance (IR), a condition associated with a pre-diabetic state, Type 2 Diabetes, and visceral adiposity, are linked to increased oxidative stress, inflammation and endothelial dysfunction (74). Inflammatory cytokines, IL-6 and TNF- α , are released from adipose tissue where their effects include inhibition of insulin signaling, stimulation of hypertriglyceridemia, and endothelial activation (74). Elevated levels of free fatty acids (FFA) have been shown to interrupt insulin transduction signaling and have been associated with the insulin resistant states often found in the obese and type 2 diabetes (75). This is of concern because insulin aids in the facilitation of glucose into muscle tissue, muscle glycogen synthesis being one of the primary pathways for glucose metabolism. It has been shown that there is an increased release of non-esterified fatty acids (NEFAs), also known as FFA, into circulation in obese individuals and that this release persists even in a hyperinsulemic state (75).

FFAs are derived through lipolysis of stored triacylglycerol (TAG) in adipose tissue and an excessive influx of FFA into muscle tissue leads to insulin resistance (75). Because there is a strong relationship between increased intramuscular TAG stores and insulin resistance, it has been suggested that an increased influx of FAs and subsequent increases in levels of

intramuscular TAG inhibit glucose transport into the cell via interruption of insulin signaling (75).

Investigators have found that elevated FFA and glucose may also induce superoxide production by nicotinamide adenine dinucleotide phosphate (NADPH) oxidases in smooth muscle cells and endothelial cells cultures (76). Investigators, such as Avogaro et al., have shown that acutely elevated plasma FFA after a 120 min lipid (TAG) infusion impairs the NO-independent vasodilation (found in the smaller peripheral vasculature) as measured by forearm blood flow (FBF) in normal weight healthy individuals. NO-independent and NO-dependent (FMD at the brachial artery) endothelial function was measured at baseline and after infusion. No change was observed in the NO-dependent brachial artery after the lipid infusion. Yet, an impairment was seen in the smaller peripheral vasculature indicating that increased plasma levels of FFA inhibited K^+ channels associated with insulin-induced NO production (76). Thus an increase in plasma FFAs can decrease insulin signaling and influence vasodilation. This evidence supports the role FFA and impaired glucose uptake in elevated oxidative stress.

Over-nutrition, in general, has the potential to result in an accumulation of adipose tissue and increased adipokine production (44). As stated before, negative energy balance can be employed as a means to reduce oxidative stress and inflammation, and impact these processes. Dandona et al. (44) found a decrease in mononuclear cells and polymorphonuclear leukocytes (PMN) ROS production in overweight subjects as a result of 12 weeks of dietary restriction with a mean weight loss of 4.5 ± 2.8 kg (9.9 ± 6.2 lbs). The ROS production decreased before any significant weight loss occurred, as soon as one week after diet initiation, leading to the speculation that ROS generation is associated with energy balance status rather than adipose stores alone. Conversely, acute increases in energy intake can increase ROS production. Dandona et al. observed an increase in ROS production by MNC and PMN after administration of an oral glucose tolerance test (OGTT) whether in energy balance or after 4 weeks of diet restriction. These results indicate that regardless of weight loss, an acute ingestion of glucose, with a hyperglycemic response, was associated with the increased production of ROS (44).

The exact contributing mechanisms are somewhat unclear, yet there is a good amount of supporting evidence that increased adiposity, insulin resistance, elevated plasma free fatty acids (FFA) and glucose, and decreased antioxidant capacity (poor diet and/or elevated oxidative stress) are a few factors that play significant roles in the state of higher inflammation and

oxidative stress in obesity; this ultimately can be tied to the increase in risk of chronic disease in this population.

INFLUENCE OF DIET COMPOSITION AND PATTERNS ON OXIDATIVE STRESS AND INFLAMMATION

Dietary macronutrient composition and dietary patterns such as bingeing and meal skipping may also influence CVD risk (34). Increased ROS production from a high calorie mixed meal can stimulate oxidative stress which activates NF- κ B which then promotes production of cytokines as well as CRP by the liver (40). Studies have also shown that specific dietary components influence inflammation (11, 77). Esposito et al. investigated the effects of the Mediterranean diet on markers of inflammation in subjects with the metabolic syndrome (11). The diet included an increased consumption of whole grains, fruits and vegetables, nuts and olive oil. After two years on the diet, subjects in the intervention group had significant decreases in levels of inflammatory cytokines when compared to the control group. In the intervention group, baseline measurements for IL-6 decreased from a baseline of 2.1 to 1.4 pg/mL, and CRP from 2.8 to 1.7 mg/mL over two years. Esposito's group found improved insulin resistance and endothelial function (L-arginine test) as well. Values for HOMA¹, and Endothelial Function Score² increased from 3.7 to 2.5 and 6 to 7.9, respectively (11). Because the Mediterranean diet not only increases MUFA and PUFA, but the overall antioxidant quality of the diet as well, Esposito's group was unable to differentiate between factors that may have influenced their findings. Other studies have provided evidence of inflammation reduction through the supplementation of certain foods including tea epigallocatechin-3-gallate (flavonoid), wine, nuts, and olive oil (78-81). Interestingly, many of these foods may be found in a Mediterranean diet, a regimen which has been found to be associated with a decrease in CVD risk (82). The beneficial qualities of the Mediterranean diet have been attributed to a variety of mechanisms including preservation of vascular function as well plaque stabilization through decreased inflammation (11). The traditional macronutrient composition of this Mediterranean diet mimics current

¹ HOMA – Homeostasis model assessment: Calculated from fasting plasma glucose (mmol/L) x fasting serum insulin (μ U/mL) / 25. Low insulin sensitivity is indicated by a high score.

² Endothelial function score are designated by changes in blood pressure: 4 points are rewarded for a score between 4-5 mm Hg, etc.

American Heart Association (AHA) recommendations: 55% / 30% / 15% (carbohydrate/fat/protein) (83), unlike the typical American diet.

Some investigators found providing meals in a regular meal pattern improved antioxidant status in subjects (17). Cao et al. (17) found ORAC measures showed improvements in the antioxidant capacity of subjects' serum regardless of treatment. These findings were attributed to an effect of the study design, possibly from eating regularly spaced portioned meals during the controlled feeding periods (17), and not to changes in diet composition. Efforts to decrease health risk in obese populations should consider macronutrient composition as well as meal pattern.

Many studies have looked at the effects of antioxidants on health outcomes. Endogenous and exogenous antioxidants are fundamental components of the body's defense against oxidative damage caused by free radicals and ROS. Exogenous antioxidants are acquired in the diet chiefly through the consumption of fruits, fruit products, vegetables, and whole grains. These foods contain vitamins, minerals, and phytochemicals, all of which play roles in protecting the body's tissues from oxidative injury. Individuals that are overweight or obese who exhibit elevated oxidative stress and inflammation only advance their risk for chronic disease by pairing their increased adiposity with a low-antioxidant rich diet. Studies have shown some beneficial outcomes of antioxidant supplementation in the obese and other populations that exhibit similar levels of oxidative stress, such as smokers (84). Vitamins E and C (Table 3), flavonoids, omega-3 (n-3 fatty acids), α -lipoic acid, and others have been a few of the means utilized in investigations aimed at lowering biomarkers of oxidative stress (10, 78, 85, 86). An increased intake of these antioxidant nutrients in at risk individuals would likely decrease oxidative stress through enhanced radical scavenging, and thus decrease inflammatory responses and improve CVD risk. Since weight loss can be challenging for a good number of obese individuals, increasing antioxidant defenses is a practical and valid proposition.

Many investigations have examined the effects of single whole foods and beverages on CVD risk factors in order to better characterize the effect of antioxidants and other phytochemicals on health. Some studies have utilized flavonoids as a means to supplement the body's antioxidant defenses. These studies have included: wine, juices, fruits, vegetables, and whole grains (30, 81, 87). Of particular interest are the studies that have investigated grapes and grape products, and their effects on CVD risk factors and clinical outcomes (29, 33, 34). This has

been done in a variety of populations ranging from healthy to those with chronic disease with varying results. To date, dried grapes or raisins have not been investigated as a potential means of reducing oxidative stress in any populations, healthy or otherwise. No research has been published on the effects of raisins on markers of oxidative stress, inflammation and endothelial function in at risk populations. Because of this known association between nutrient intake and the condition of obesity as they are related to increased risk of CVD, it is safe to assume that interventions that affect weight outcomes or nutrients that improve oxidative status are warranted.

OXIDATIVE STRESS, INFLAMMATORY, AND ENDOTHELIAL RESPONSES TO A MEAL CHALLENGE

Investigators have incorporated acute meal challenges as tools for stimulating oxidative stress in normal and obese populations (Table 3, 4). Acute meal challenges as a practical test of oxidative stress, inflammation, and endothelial function are appropriate because the outcomes have real life applications. Some investigations utilize bolus feedings (glucose or lipid) to induce oxidative stress (44, 76, 88). These types of studies are not representative of normal dietary habits, where individuals usually consume mixed macronutrient meals. Type 2 diabetics have been an important population to study due to the incidence of CVD associated with this chronic disease. Dietary pattern has been shown to influence markers of glucose control in diabetics. For example, Regular meal patterns consisting of 3-6 regularly spaced meals per/day with portion control has been shown to lower the proportion hb_{A1c} in diabetic populations (89).

As stated before, the typical western pattern of eating usually includes meal skipping and/or binging. Investigators have shown that consuming a high energy meal (>800-900 kcal), particularly a high fat meal (>50% fat) can induce an inflammatory, oxidative stress, and endothelial dysfunction responses in healthy and unhealthy populations (8, 90). Most investigators have attributed this response to postprandial increases in metabolites, such as FFA, TAG, and glucose. These responses are more robust in obese and diseased populations (8). Brook et al. demonstrated that CRP was significantly correlated with FFA levels, and that an increase in plasma FFAs was associated with an increase in markers of oxidative stress in obese populations (51).

Endothelial function can be compromised in healthy subjects as well as those with known disease. Nappo et al. (8) utilized a high-fat and high-carbohydrate meal challenge in the evaluation of endothelial activation in healthy and Type 2 Diabetic subjects (Table 5). Healthy and diabetic subjects exhibited elevated levels of inflammatory cytokines and adhesion molecules after the high-fat meal. These increases were also accompanied by significant increases in plasma TAGs. This relationship persisted in healthy subjects even after antioxidant supplementation, where a significant correlation was found between elevated plasma TAG and TNF- α . In Nappo's investigation, it is important to note that the diabetic subjects had significantly elevated baseline plasma levels of inflammatory markers, adhesion molecules, HbA_{1C}, glucose, and serum TAG, as well as decreased plasma HDL compared to the healthy subjects. In diabetic subjects, markers of inflammation and endothelial activation were significantly reduced after supplementation with antioxidants (8). These findings suggest a postprandial hypertriglyceridemia and hyperglycemia condition is amplified in the diabetic subjects. It was also demonstrated that antioxidants lessened this effect suggesting an oxidative mechanism in endothelial activation. Finally, the increase in these markers in diabetics may help explain the increased risk for CVD in this population.

The importance of including healthy subjects in these investigations is to establish a reference point for postprandial responses without the complications of a diseased state. Bae et al. studied the affects of a high fat vs. low fat meal challenge in 20 healthy young female subjects. The measures included serum analysis for TAGs, PMA-activated leukocytes for superoxide production, and endothelial function by FMV(72) . Measurements were taken at baseline and 2 hrs postprandial. The investigators found that there was a significant increase in serum TAGs in the high fat meal group. Superoxide production by PMA-activated leukocytes was significantly greater at 2 hrs postprandial and when compared to low-fat meal values. FMD values were significantly lower in the high-fat group at 2 hrs postprandial vs. baseline, as well as when compared to the low-fat values. A significant negative correlation was found between changes in superoxide production and decreases in FMD values. These findings support the suggestion that a high-fat meal challenge increases oxidative stress which then induces endothelial dysfunction (72).

Marchesi et al. also demonstrated that postprandial hyperlipidemia impaired endothelial function in seven healthy men assessed by FMD (70). These effects were seen regardless of the

MUFA, PUFA, and small amounts of vitamin E used in the rice oil used as the fat load in this study. Similar results were found by Vogel et al. when ten healthy subjects consumed a 900 kcal high-fat or low-fat meals (91). The investigators found that postprandial TAG increased significantly, as well as a transient decrease in FMD values. Overall, these findings further demonstrate a relationship between the blood lipid response and the magnitude of endothelial dysfunction following a meal.

The mechanism by which elevated levels of TAG and FFA influence oxidative stress, inflammation, insulin resistance and endothelial dysfunction are elusive. Activation of key enzymes such as NADPH oxidase in the endothelium and smooth muscle cells have been implicated as a mediator of these effects (46). Other enzymes known to be involved in cellular oxidant production include, xanthine oxidase, myeloperoxidase, and lipoxygenase (92). Other mechanisms proposed involve nuclear factor kappa B (NF- κ B). NF- κ B is a nuclear transcription factor that is involved in the regulation of gene expression of factors associated with immunity, inflammation, coagulation, and endothelial function (88). NF- κ B induction is influenced by a variety of factors including TNF- α and oxidized LDL (88). Some investigators have found oxidative measures to be associated with the activation of NF- κ B, possibly mediated by an increase in blood lipids (24). These findings support the increase in oxidative stress markers in response to elevated blood lipids.

MEAL COMPOSITION INFLUENCE ON INFLAMMATION, OXIDATIVE STRESS AND ENDOTHELIAL DYSFUNCTION

Many investigations have explored the effects of single vitamin antioxidants such as vitamin E (α -tocopherol) and C (ascorbic acid) on disease risk in humans (8, 91). The literature results are often contradictory. Vitamin E and C often work synergistically, in that ascorbic acid has the ability to reduce oxidized α -tocopherol and thus regenerate α -tocopherols' antioxidant capabilities (10). Both α -tocopherol and ascorbic acid have the ability to directly scavenge free radicals and thus are indispensable components of antioxidant systems in humans (21). It is because of the ROS quenching characteristics that these particular compounds have that makes them such attractive candidates for research involving oxidative stress and antioxidant interventions. However, studies that have utilized whole foods containing natural sources of

antioxidants have the advantage of modeling dietary interventions without single nutrient supplementation. Because there is a mixture of compounds with antioxidant properties in whole foods that may have unidentified benefits other than the more thoroughly investigated antioxidants (α -tocopherol and ascorbic acid), whole foods may be a superior method of “supplementation”.

Interventions utilizing whole food interventions have the advantage of real life applications. Since most people eat a mixed and varied diet, it is relevant to investigate whole foods as a means of CVD risk reduction. Cao et al. had 36 healthy subjects consume one of two diets (A and B) for 15 days during a residency period, each in a cross-over design composed of either ten servings of fruits (including grapes and raisins) and vegetables, or ten servings plus two additional servings of broccoli for days 6-11. Oxygen radical absorbance capacity (ORAC) was measured at baseline, days 6, 11, and 16 (17). The study found that there was a significant increase in ORAC from baseline of 54 to 95 $\mu\text{mol Trolox equivalent/L}$ as well as significant increases in α -tocopherol amounting to 1.7-3.7 $\mu\text{mol/L}$ in both the diets. A significant correlation was found between increases in ORAC and α -tocopherol concentrations in younger subjects in diet A, but not in older subjects consuming the same diet. There was no correlation found in those subjects, young or old, consuming diet B. Given that there was a significant increase in ORAC in both diets, Cao et al. was able to demonstrate that the changes could not be attributed to the increases in α -tocopherol alone (17). Additionally, since there was an increase in ORAC with both treatments, improvements may be attributed to the controlled feeding period (regular meal pattern) as well.

Studies have investigated the effects of grape products and markers of oxidative stress (19, 34, 93). O’Byrne et al. (34) investigated the effects of chronic consumption of Concord grape juice, a good source of flavonoids (catechin, epicatechin, quercetin, and anthocyanins), on plasma ORAC, serum total and conjugated phenols, as well as markers of oxidative stress, LDL oxidation and urinary F_2 -isoprostanes. The researchers compared the consumption of 10 mL/kg of juice supplementation each day with the effects of a daily 400IU α -tocopherol supplement. The juice supplemented group had similar increases in ORAC and decreased LDL susceptibility to oxidation to those supplemented with α -tocopherol. These findings signify an overall increased antioxidant effect in the serum. Significant increases in plasma flavonoids after 10-12 hours of fasting allowing for an extended period of protection (34).

Some investigators looked at the effects of grape products on postprandial measures of oxidative stress (24, 94). Blanco-Colio et al. (24) investigated the effects of red wine, a good source of antioxidant compounds (resveratrol, quercetin and catechin), on peripheral blood mononuclear cells (PMBC) of healthy subjects after a high-fat meal. Subjects that were supplemented with the red wine exhibited an attenuation of NF- κ B activation regardless of increases in TAG and VLDL (24). These findings provide a link between antioxidant interventions on a transcription factor known to be involved in mediating inflammatory processes. This information could have special applications in populations that have elevated blood TAG and increased inflammation, such as the overweight, obese, and those with chronic disease.

FLAVONOIDS AND RAISINS

The extensive use of grapes and grape products in research is primarily due to their antioxidant potential attributed to flavonoids. There have been over 4000 different flavonoids found to be naturally occurring in foods of plant origin (26). These compounds serve to protect plants from stresses that would threaten their survival in nature. Human consumption of foods with high flavonoid content has been associated with a decreased risk for chronic diseases including CVD (15). The possible mechanisms that flavonoids may effect include: direct scavenging of some radical species; suppression of lipid peroxidation by recycling other antioxidants (α -tocopherol) by donating an 'H' to α -tocopherol; or chelation of pro-oxidant metal ions (iron and copper), that prevent free radical formation; protection of DNA; deactivation of carcinogens; inhibition of the expression of mutated genes that promote carcinogenesis, as well as promoting the detoxification of xenobiotics (26).

Flavonoids may play a role in modifying enzymatic systems involved in ROS generation in the vasculature as seen in the literature investigating the effects of wine and grape juice on vascular function (24, 34). Flavonoids have the unique ability to function in both the aqueous and lipid compartments (26). Structural diversity gives them an advantage over nutrient antioxidants that may be limited to one compartment such is the case with lipid soluble α -tocopherol and water soluble ascorbic acid. The flavonoids in grapes and grape products have been of great interest because of their ability to prevent LDL oxidation *in vitro*, which is

associated with atherosclerotic plaque formation (93). The most powerful effectors have been found to be quercetin and epicatechin, both of which are found in grapes, including raisins (27).

Grape products are good sources of polyphenolic compounds. These compounds not only exhibit antiviral and anticancer properties, they have also been associated with a reduced risk of CVD (27). Grapes and wine have several compounds (Table 5) that may be lost in the process of drying. The process of drying is done by allowing the grapes to dry on paper in the sun between vineyard rows for 2-3 weeks. The grapes are dried to a final moisture content of 9-16%, which increases slightly after washing and packaging, to 15-18% (27). According to Karadeniz et al., raisins and grapes contain various phenolic and flavonols. Of the phenolic compounds (caftaric and coumaric acids), raisin concentrations decreased as a result of drying from 103.4 to 39.6 mg/kg and 39.8 to 6.7 mg/kg of sample, respectively. Oxidized cinnamics increased in raisins and were not present in fresh grapes. Of the flavonols, rutin, quercetin glycosides, and kaempferol glycosides, rutin increased from 0.9 to 5.7 mg/kg, as well as the glycosides combined, from 45.2 to 76.9 mg/kg of sample. Finally, Arts et al. (28) found concentrations of flavon-3-ols, catechin and epicatechin in raisins, where Karadeniz's group did not analyze these compounds. Of the compounds in raisins, quercetin (primarily found in glycoside form) is the most thoroughly investigated because it is found in many commonly eaten foods, such as grapes, apples, and onions (27, 95). Interestingly, the compounds believed to exhibit vasodilatory effects, the proanthocyanidins ((+)-catechin and (-)-epicatechin) (96), are found in grape seeds, grapes, wine and raisins.

In order for flavonoids to have any type of bioactivity in the body they must be absorbed into circulation. Several investigators have looked at the bioavailability of flavonoids and factors that may inhibit their absorption (26, 82). Quercetin naturally occurs in two forms, quercetin glycoside and rutinoid. The glycosidic form is readily absorbed in the small intestine after the removal of the glycoside, followed by conjugation to glucuronides and/or sulfates ((82, 97)). The rutinoid form is absorbed in the colon and is not as efficiently absorbed as the glucoside form (82, 97). Recent investigations have found that quercetin in red wine is not as well absorbed as similar quantities found in onions and black tea (98). Onions were found to have a better absorption and it was speculated that this was due to the quercetin existing primarily in a glycosidic form in this food (26). There has not been a lot of data collected on factors that inhibit

the absorption of quercetin or other flavonoids. For example, some studies have stated that milk inhibits the absorption of quercetin, Hollman et al. found this was not true (26).

In order to investigate the effects of raisins, a comparable amount of phenolic compounds need to be consumed. O'Bryne et al. (34) used 10 mL/kg of Concord grape juice which corresponded to 560 mg phenolic equivalents/L. 90 grams of raisins is estimated to have approximately 359 mg of phenolic equivalents (32) and thus it is speculated that this amount will have similar health benefits as was found by O'Bryne's group.

SUMMARY

Obesity increases risk for CVD. This may be partly explained by a sustained inflammatory state brought about overwhelming of the body's antioxidant defense system. This is evident in the literature that supports the findings that elevated markers of oxidative stress, inflammation, and endothelial dysfunction are associated with obesity, particularly visceral adiposity. The use of antioxidant rich foods may help ameliorate these negative health effects by increasing the body's antioxidant pool and thus decreasing inflammation brought about by increased generation of ROS. Furthermore, an increase in antioxidants may help preserve the bioavailability of NO and thus improve vasodilation capacity and thus lower the risk of atherosclerotic CVD progression. The consumption of raisins, a good source of flavonoids with antioxidant properties, as a functional food may be an inexpensive and easily accessible method of intervention to improve oxidative stress, inflammation and endothelial dysfunction and thus the health of overweight individuals.

Table 2.1.: Comparison of Serum Markers: Lean and Obese

Marker	CRP (mg/L)	IL-6 (pg/mL)	Urinary F₂- isoprostanes (ng/mg Cr)(24hr)	TAG	Glucose	HbA_{1c} (%)	Insulin (μU/mL)	sICAM- 1(ng/mL)	sVCAM- 1(ng/mL)
Normal Values	Low risk: < 1.0 Average risk: 1.0 to 3.0 High risk: > 3.0 (99)	0.69, 1.0, 1.3 (100- 102)	3.3 (61)	≤150 mg/dL (1.7mmol/L) (103)	≤100 mg/dL (≥6.1 mmol/L) (103)	2.2- 4.8 (103)	8.8, 11.6 (101, 104)	300.4- 319.9 (79)	436.2- 456.8 (79)
Values in overweight/ obese populations	3.2-3.4, 5.98 (49, 105)	4.1-4.3, 2.19, 3.08 (41, 49, 105)	5.0 (61)	142 mg/dL, 1.48 mmol/L (49, 105)	105-106 mg/dL, 5.4 mmol/L (49, 105)	5.8 (100)	14, 14 (49, 105)	553-603 (6)	789-796 (6)

Abbreviations: CRP (C Reactive Protein), IL-6 (Interleukin 6), TAG (Triacylglycerol), Hb_{A1C} (Hemoglobin A1C), sICAM-1 (soluble Intracellular adhesion molecule 1), sVCAM-1 (soluble Vascular adhesion molecule 1)

Table 2.2.: Markers of oxidative stress in the obese

Source	BMI		Markers (ox stress)	
	Normal	Obese	Normal	Obese
Dandona et al. 2001(44)	22.5 ± 1.0	40.7 ± 9.4	>TBARS 1.29 ± 0.12 (μmol/L) >9-HODE 1.97 ± 0.84(μmol/L) >13-HODE 2.04 ± 0.72 (μmol/L) >Carbonylated proteins 0.60 ± 0.10 (μmol/mg) >o-Tyrosine/phenylalanine ratio 0.28 ± 0.02 (μmol/mol) >m-Tyrosine/phenylalanine ratio 0.27 ± 0.03 (μmol/mol)	>TBARS 1.68 ± 0.17 (μmol/L) >9-HODE 7.10 ± 3.88 (μmol/L) >13-HODE 6.67 ± 3.85 (μmol/L) >Carbonylated proteins* 1.39 ± 0.27 (μmol/mg) >o-Tyrosine/phenylalanine ratio 0.42 ± 0.03 (μmol/mol) >m-Tyrosine/phenylalanine ratio 0.45 ± 0.04 (μmol/mol)
Stojiljkovic et al. 2002(61)	22.7 ± 0.5	35 ± 2.0	Urinary <i>F</i> ₂ -isoprostanes (ng/mg Cr)(24hr) 3.3 ± 0.3	Urinary <i>F</i> ₂ -isoprostanes (ng/mg Cr)(24hr) 5.0 ± 0.5
Higashi et al. 2003(106)	<u>Normal</u> 22.9 ± 1.8 <u>Low</u> 17.9 ± 0.5	<u>Overweight</u> 27.5 ± 1.4 <u>Obese</u> 34.6 ± 3.7	8-OHdG (ng/ml) <u>Normal</u> 1.14 ± 0.80 <u>Low</u> 2.7 ± 1.8 MDA-LDL <u>Normal</u> 58.8 ± 21.2 <u>Low</u> 76.5 ± 30.2	8-OHdG (ng/ml) <u>Overweight</u> 2.44 ± 1.65* <u>Obese</u> 3.91 ± 2.36* MDA-LDL <u>Overweight</u> 79.6 ± 25.7* <u>Obese</u> 83.4 ± 29.7*
Ohmori et al. 2005(107)	<u>Normal</u> 18.5-24.9 <u>Low</u> <18.5	<u>Overweight</u> 25.0-29.9 <u>Obese</u> >30.0	8-iso-PGF _{2α} (plasma - GCMS) <u>Normal</u> 19.96 ± 0.21 <u>Low</u> 18.94 ± 0.68	8-iso-PGF _{2α} (plasma - GCMS) <u>Overweight</u> 20.46 ± 0.29 <u>Obese</u> 20.98 ± 0.77

BMI classifications: <18.5 = low or underweight, 18.5-24.9 = normal, 25-29.9 = overweight, 30-34.9 = obese, >35 = morbidly obese

Abbreviations: Thiobarbituric acid-reactive substances (TBARS), 8-Hydroxy-2'-deoxyguanosine (8-OHdG), 9-hydroxyoctadecadienoic acid (9-HODE), 13-hydroxyoctadecadienoic acid (13-HODE), malondialdehyde-modified low-density lipoprotein (MDA-LDL). Gas Chromatography/mass spectrometry (GCMS)

* Indicates statistical significance between normal BMI and obese

Table 2.3.: Meal challenges: Composition

Data source	Subject	Meal	Meal Composition	Subject Amount	Intervention
Djousse et al, 1999(94)	13 Healthy subjects	Burger king: Whopper w/cheese and Fries	CHO: 19% PRO: 20% FAT: 48%	0.8 g/kg fat 15 Kcal/kg	Red wine: 3mL/kg (cabernet sauvignon, CA) = 13% of meal energy
Plotnick et al, 2003(108)	Healthy, subjects	McDonalds Egg McMuffin, Sausage McMuffin, 2 hash patties and non-caffeinated beverages	FAT: 50% = 50g SFA: 14g CHOL:225mg	899.49 kcal	Juice Plus, Juice Plus and Vineyard JP=dried juice conc. V=multiplying Including grape skin and seed
Vogel et al. 2000 (22)	10 healthy subjects	Comparison of 5 different high-fat meals	FAT: 50% = 50g	900 kcal	Vitamins C and E Salad w/tomatoes
Vogel et al. 1997 (91)	10 healthy subjects	McDonalds Egg McMuffin, Sausage McMuffin, 2 hash patties and non-caffeinated beverage	FAT: 50% = 50g	900 kcal	none
Marchesi et al, 2002(70)	7 healthy subjects	Sandwich – 30g white bread (~2 slices, 100g tomatoes, mayo prepped w/65g rice oil, ½ egg yolk = 700Kcal	CHO: 25% = 43.75g PRO: 5% = 8.756g FAT: 70% = 54.44g	700Kcal (Per m ² body surface area)	none
Bae et al. 2001 (72)	20 healthy subjects	Rice (110g), Korean BBQ (100g), egg (20g), milk (200ml), oil (8g), mayo (25g), veg.(50g)	CHO: 24.91% = 50g PRO: 15.29% = 30.7g FAT: 59.9% = 53.40g	803 kcal	none
Nappo et al. 2002 (8)	20 newly diagnosed Type 2DM 20 healthy controls	2 sausages (80g), 6 bread slices (90g), 1 sm. Egg (40g), butter (15g), olive oil (5g)	CHO: 28.5% PRO: 12.3% FAT: 59.2%	760 kcal	Vit E (800 IU) Vit C (1,000 mg)

Abbreviations: CHO (carbohydrate, PRO (protein), kcal (calories), vit (vitamin), Type 2DM (Type 2 Diabetes Mellitus)

Table 2.4.: Metabolic and Endothelial Responses to Meals

<i>Data source</i>	<i>Subject</i>	<i>Meal Composition</i>	<i>Subject Amount</i>	<i>Markers of inflammation/oxidative stress</i>	<i>TAG and Glucose</i>	<i>Intervention</i>
Ceriello et al. 2004 (90)	30 newly diagnosed Type 2DM 20 healthy controls	High Fat: CHO: 2.9% PRO: 3.4% FAT: 96.4%	~700 kcal/m ²	2DM: ↑sICAM-1, sVCAM-1, E-selectin, nitrotyrosine ³ , Healthy: ↑sICAM-1, sVCAM-1, E-selectin, nitrotyrosine	2DM: ↑ TAG, ↑Glucose Healthy: ↑ TAG, ↑Glucose	2DM: 3-6 d simvastatin had ↔TAG, but *↓basal sICAM-1, sVCAM-1, nitrotyrosine – no change on postprandial TAG from baseline test meal.
		High CHO: 75 g glucose (OGTT)		2DM: *↑sICAM-1, sVCAM-1, E-selectin, nitrotyrosine Healthy: *↑sICAM-1, sVCAM-1, E-selectin, nitrotyrosine	2DM: ↔TAG, ↑Glucose (1, 2, 3 hr) Healthy: ↔TAG, ↑Glucose (1 hr)	2DM: : 3-6 d simvastatin ⁴ had ↔TAG, but *↓basal sICAM-1, sVCAM-1, nitrotyrosine ↔TAG
		High CHO + High Fat: CHO: 2.9% PRO: 3.4% FAT: 96.4% 75 g glucose (OGTT)		2DM: ↑sICAM-1, sVCAM-1, E-selectin, nitrotyrosine (2 hr) Healthy: ↑sICAM-1, sVCAM-1, E-selectin, nitrotyrosine (1 hr)	2DM: ↑ TAG, ↑Glucose (1, 2, 3 hr) Healthy: ↑ TAG, ↑Glucose (1 hr)	2DM: : 3-6 d simvastatin had ↔TAG, but *↓basal sICAM-1, sVCAM-1, nitrotyrosine – no change on postprandial TAG from baseline test meal.
Nappo et al. 2002(8)	20 newly diagnosed Type 2DM 20 healthy controls	High Fat: CHO: 28.5% PRO: 12.3% FAT: 59.2%	760 kcal	2DM: **↑TNF-α, IL-6, sICAM-1, sVCAM-1 Healthy: *↑TNF-α, IL-6, sICAM-1, sVCAM-1	2DM: **↑ TAG, ↑Glucose Healthy: ↑TAG ↔glucose	2DM: w/ vit. C&E ↑ remained but reduced for TNF-α, IL-6, sICAM-1, sVCAM-1 Healthy: w/ vit. C&E ↑ IL-6, sICAM-1, sVCAM-1 no longer significant – except *↑TAG
	20 newly diagnosed Type 2DM 20 healthy controls	High CHO: CHO: 72.9% PRO: 6.5% FAT: 20.6%	760 kcal	2DM: **↑TNF-α, IL-6, sICAM-1, sVCAM-1 Healthy: ↔TNF-α, IL-6, sICAM-1, sVCAM-1	2DM: ↑Glucose Healthy: ↔TAG, ↔glucose	2DM: w/ vit. C&E ↑ remained but reduced for TNF-α, IL-6, sICAM-1, sVCAM-1 Healthy: ↔ no change

*Statistical significance between baseline and postprandial. **Statistical significance between postprandial 2DM & Healthy subjects. ↔ = No Change. Abbreviations: TAG (Triacylglycerol), Hb_{A1C}, sICAM-1 (soluble Intracellular adhesion molecule 1), sVCAM-1 (soluble Vascular adhesion molecule 1), TNF-α (Tumor Necrosis Factor alpha), Type 2DM (Type 2 Diabetes Mellitus), vit. (Vitamin)

³ nitrotyrosine is a product of peroxynitrate (product of NO and O₂⁻) nitration of tyrosine residues – marker of oxidative damage

⁴ Simvastatin is a pharmaceutical lipid-lowering drug (statin) proposed to exhibit antioxidant properties

Table 2.5.: Raisin and Grape composition

Source Data	Food	Flavan-3-ols	Procyanins -oligomeric procyanidins (Proanthocyanidins)	Flavonols	Resveratrol	Rutin (quercetin-3-rutinoside)	Cinnamic Acid Derivatives	Oxidized cinnamics
Karadeniz et al, 2000(27)	Grapes	(+)-Catechin (-)-Epicatechin	Dimers B1-B4	Quercetin glycoside A&B Kaempferol glycoside A	Resveratrol	Rutin	<i>cis</i> -coumaric acid and coumaric acid	none
	<u>Raisins</u> (Thompson seedless grapes)	none	none	<u>Quercetin glycoside A</u> <u>Kaempferol glycoside A&B</u>	none	<u>Rutin</u>	<u>coumaric acid</u>	<u>cinnamic A and B</u>
USDA Database (109)	Seedless <u>Raisins</u>	<u>(+)-Catechin</u> <u>(-)-Epicatechin</u>	none	none	none	none	none	none
	Red Grapes (raw)		Monomers, dimers, trimers, 4-6mers, 7-10mers, polymers	none	none	none	none	none
O'Byrne et al, 2002(18)	Grape juice (Concord)	(+)-Catechin (-)-Epicatechin	anthocyanidins	Quercetin	none	none	none	none
Arts et al, 2000 (28)	<u>Raisins</u>	<u>(+)-Catechin</u> <u>(-)-Epicatechin</u>	none	none	none	none	none	none

CHAPTER 3

Value of Raisins for Reduction of Oxidative Stress, Endothelial Dysfunction, and Inflammation in Obesity

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ABSTRACT

Aim: An elevated state of inflammation initiated by oxidative stress is associated with an increased risk for cardiovascular disease (CVD). The objective of this study was to determine effects of daily consumption of raisins on markers of inflammation, oxidative stress and endothelial activation in response to an acute high-fat meal in obese individuals.

Methods: Seventeen overweight men and women consumed Thompson seedless raisins or placebo (264 kcal/d) daily for 14 d in a randomized cross-over design while following a low-flavonoid, weight-maintenance diet. Four high-fat (53% fat) meals were consumed with the respective treatment pre and post interventions. Measures at fasting and 2, 3 and 4 hours postprandial included markers of oxidative stress (urinary 8-isoPGF_{2α}; serum Oxygen Radical Absorbance Capacity, ORAC), inflammation (serum C-reactive protein, CRP; interleukin-6, IL-6), endothelial function (serum soluble intercellular adhesion molecule-1, sICAM-1; soluble vascular adhesion molecule-1, sVCAM-1), and metabolic measures (free fatty acids (FFA), triacylglycerol (TAG), glucose, insulin).

Results: Urinary 8-isoPGF_{2α} decreased 22% and ORAC increased 3% pre to post interventions combined. Postprandial metabolic responses differed by gender, males surpassed females for several measures: FFA, triacylglycerol, glucose, and sVCAM-1. Neither the meals nor treatment with raisins had any noteworthy influence on fasted measures of inflammation or endothelial dysfunction.

Conclusions: Acute high fat meal consumption did not result in evidence of inflammation or oxidative stress in these relatively healthy, overweight individuals. Providing all food in regular pattern may have reduced measures of oxidative stress. Gender influenced metabolic responses to meals; males had a greater postprandial response in metabolic measures than females.

Keywords: Postprandial response, high fat meal, antioxidants, flavonoids, cytokines, endothelial activation, ORAC

INTRODUCTION:

Overweight and obesity are associated with increased health risk for chronic diseases such as diabetes, cancer, and cardiovascular disease (CVD) (1,2): Chronically elevated oxidative stress and inflammation, illustrated by increases in oxidatively modified macromolecules (DNA, lipids, carbohydrates, and proteins) (3), serum interleukin-6 (IL-6) (4,5), C-reactive protein (CRP) (4,5), and markers of endothelial activation (5), are related to adiposity and predict higher risk of cardiovascular disease. In fact, recent studies find that serum CRP is a strong risk factor independent of blood lipids and other markers. In the Jupiter Study, subjects with elevated inflammation (serum CRP > 2 mg/L) but not hyperlipidemia responded to a statin drug with a reduction in serum CRP and more than 50% reduction in cardiovascular events (6).

Several studies have shown that high fat meals acutely increase inflammatory or oxidative stress markers. For example, Nappo et al. found that a high-fat meal increased plasma concentrations of inflammatory markers tumor necrosis factor- α (TNF- α), IL-6, CRP, sICAM-1, and sVCAM-1 in type 2 diabetics as well as normal controls when compared to an isocaloric low-fat meal (7). The addition of antioxidants E and C ameliorated these effects suggesting that this response is linked to the ability of the body to quench free radicals. As much of the day is spent in the postprandial state, it is of interest to determine whether addition of foods containing antioxidant compounds to the daily diet of overweight individuals could reduce their basal as well as postprandial inflammatory state.

Interventions using the Mediterranean diet have documented a reduction in chronic inflammation (8-10). However, as this diet recommends increasing a variety of foods with antioxidant properties (fruits, vegetables, wine), it is unclear what particular component of the diet is most important in reducing inflammation and risk of disease.

Fruits are known to have a variety of polyphenolic constituents such as flavonoids, phenolic acids and tannins (11). In epidemiological studies, such as the Zutphen Elderly Study and the Rotterdam study, investigators demonstrated that individuals in the highest tertile for flavonoid consumption had a 58% reduced risk of CVD compared to tertiles with lower consumption of flavonoids (12), and that dietary flavonoids from sources such as tea consumed over a number of years was associated with a lowered CVD risk (13). Although there is evidence that a flavonoid-rich diet is associated with reduced health risk, it is still unclear whether or not this is due to a reduction in oxidative stress. Some investigations have explored

the effects of total fruit and vegetable intake on markers of oxidative stress. Kim et al. (11) showed an increase (41%) in erythrocyte superoxide dismutase (SOD) enzyme activity in normal weight, non-smoking young females after subjects consumed a phenol-depleted diet for 6 days, thus indicating the compensation of endogenous antioxidant systems in the absence of adequate dietary antioxidants. Daily consumption of one high flavonoid food, Concord grape juice, increased lag-time in LDL susceptibility to oxidation (14). Similar improvement in oxidative state were seen in an investigation by Zern et al. (10) of the effects of a daily consumption of lyophilized grape powder supplement to reduce TNF- α as well as urinary measures which were significantly by the intervention.

Increases in oxidative stress and chronic inflammation are also associated with poor endothelial function (15), impaired vasodilation being the key step in CVD progression. However, the link between dietary components, oxidative state and endothelial function remains unclear. Several investigations have looked at the effects of flavonoid rich foods and oxidative mechanisms as well as vasodilatory capabilities. For example, Vogel et al. saw a 71% improvement in flow mediated vasodilation (FMD) when vitamin E and C were added to a high fat meal (50% fat) of olive oil and bread (16). These findings suggest an oxidative mechanism may disrupt normal vascular function.

Studies showing health benefits of wine and grape juice suggest that raisins may have similar benefits. Wines, grapes, and raisins are high in flavonoids such as resveratrol (wine and grapes only), quercetin, kaempferol, (+)-catechin, and (+)-epicatechin (14,17,18). Seedless raisins are one of the Key Foods identified by the United States Department of Agriculture (USDA) (19) suggesting they are a significant source of nutrients pertinent to health concerns in the US population (19). Among other important nutrients, raisins contain 359.5 mg total phenols per 90g serving compared to 142.2 mg for green and 198.5 mg for red grapes (also 90 gram servings) (43). To date, no investigations utilizing seedless raisins as a dietary intervention to reduce inflammation have been conducted.

This study investigated the effects of acute and chronic consumption of seedless raisins on markers of oxidative stress, inflammation, and endothelial activation, in the fasted state and in response to an acute high-fat meal challenge in overweight and obese subjects. The purpose of this study was to help determine whether a dietary intervention utilizing a whole food source of

phenolic compounds would be capable of disconnecting obesity from some of the associated health problems.

METHODS:

Subjects:

Twenty overweight men or women ($\text{BMI} \geq 26 \text{ kg/m}^2$), between the ages of 18 and 40 years old, volunteered to participate after being informed of all risks associated with the investigation. All subjects completed a questionnaire on current and past health history, dietary allergies, as well as a written informed consent prior to participation in the study. All investigative procedures were reviewed and approved by the Human Subjects Institutional Review Board of Virginia Tech.

Subjects were asked to maintain their weight and activity level, as well as refrain from taking any dietary supplements and anti-inflammatory medications 2 weeks prior to and for the duration of the study. Individuals with diagnosed cardiovascular disease, diabetes, hypertension, tobacco use, lack of stable weight ($\pm 0.5 \text{ kg}$) over last 3 months, heavy exercise ($> 120 \text{ min/wk}$ of moderate to intense activity), and individuals with pertinent food aversions or allergies were excluded.

Qualifying subjects had fasting blood samples taken for analysis of CRP 2 weeks prior to starting the study. Any subjects with values greater than 10 mg/L were interviewed in order to dismiss any evidence of acute infection or illness. Any subjects with values greater than 20 mg/L were prohibited from participating in the investigation. One subject was excluded based on this exclusion criterion.

Study Design:

A randomized, cross-over design was used in order to have subjects undergo both treatments. The duration of the study for each subject totaled 56 days: Days 1-14 consisted of a lead-in low-flavonoid diet modeled after diets by O'Byrne et al. (20) and Peterson et al. (21), followed by 14 days (15-28) of low flavonoid diet plus the first intervention, 14 days (29-42) of washout between interventions, and finally 14 days (43-56) of the alternate intervention (Figure 1). Assignment to initial intervention was counterbalanced so that half of subjects did the raisin intervention first and the other half did placebo treatment first.

In order to insure a low flavonoid diet and consumption of the treatments in the second week of each intervention (days 22-28 and 50-56), subjects were provided with all their daily food by investigators. Subjects arrived fasted at 7AM. Following the morning body weight measurement, all subjects consumed a low flavonoid and low antioxidant breakfast in the lab, followed by a 6PM dinner meal. Lunches were take-away meals and were packed in a portable cooler. Before and after serving, all food portions were weighed on food scales to estimate waste.

Three days of rotating menus were designed for controlled feeding periods. Meals were modeled after a standard western diet. All menus complied with American Heart Association (AHA) macronutrient recommendations of 50% carbohydrate (CHO), 30% fat, and 15% protein (PRO), and were analyzed via a food analysis program for total antioxidant vitamins C, E, and A. Menus were adjusted to accommodate multiple calorie levels; 1700, 2000, 2500, 3000, and 3500 kcal/day in order to best match subjects needs (Table 1). Subject caloric needs for weight maintenance were estimated using a modified Harris-Benedict Equation for sedentary overweight adults (American Dietetic Association - ADA). Adjustments for weight loss were made using 100 kcal low-fat carbohydrate snacks.

Subjects completed an acute meal challenge prior to and at the end of each intervention period (d 15, 28, 43, and 56). The meal challenge consisted of a slightly modified McDonald's Big Breakfast® which substituted the hash browns with an additional sausage patty, as well as 132 kcal either of raisins (45 g) or jelly candy (35 g). The total meal with the intervention contained 900 kcal with, 53% fat, 34% CHO, 14% PRO. Blood draws were taken at time 0 and times 2, 3 and 4 hours postprandial; Single void urine was collected at time 0 only.

Diet Records and Controlled Feeding Menus:

Subjects were instructed on how to properly complete a 4-day food record (3 weekdays, 1 weekend day) to be completed prior to the initiation of the low-flavonoid diet (d 1). Instructional handouts accompanied the food record for assistance, as well as the use of food models for portion size estimation. Food records were done in order to estimate subjects' habitual macronutrient profile as well as micronutrient intake. Two unannounced 24hour recalls (one week day and one weekend day) were conducted via phone interviews during the lead-in period (d 1-14) and the wash out period (d 29-40) in order to support diet compliance. All food records and menus were analyzed by Nutrition Data System for Research (NDS-R version 5.0_35), Nutrition Coordinating Center, University of Minnesota, Minneapolis, MS.

Blood Collection:

After a brief rest in the supine position, blood was taken from the arm by means of venipuncture with minimum stasis by a trained phlebotomist. After collection, blood was allowed to clot for 15 min, centrifuged at 2500 rpm for 15 minutes at 4°C, and the serum was frozen at -80°C until later analysis.

Time 0 samples were measured for Total Oxygen Radical Absorbance Capacity (ORAC_{total}), protein free serum (ORAC_{pca}) assays and serum C-reactive protein (CRP), Interleukin-6 (IL-6), soluble Intercellular Adhesion Molecule-1 (sICAM-1), soluble Vascular Cell Adhesion Molecule-1 (sVCAM-1), free fatty acids (FFA), triacylglycerol, glucose, and insulin. All measures except ORAC_{total} and ORAC_{pca} were measured in the other blood samples taken at 2, 3, and 4 hour after the meal.

Sample Analysis:

Fasting serum samples designated for ORAC_{pca} were prepared immediately according to Prior and Cao (1999), and frozen at -80°C until later analysis. Sample preparation included reacting serum protein-precipitated plasma (ORAC_{pca}) with 0.5 M perchloric acid (PCA) (1:1 v/v) to precipitate proteins. The protein-free supernatants as well as the serum for ORAC_{total} were flushed with nitrogen gas, flash frozen in liquid nitrogen and stored at -80°C until later analysis. ORAC_{total} and ORAC_{pca} were analyzed by the United States Department of Agriculture (USDA) Jean Mayer Human Nutrition Research Center on Aging Antioxidants Research Lab, located at Tufts University, Boston, MA. according to modified methods of Ou et al. (22) and Cao et al. (23). Briefly, the ORAC assay provides an integrated and quantitative determination of "total antioxidant capacity" by employing the area under the curve (AUC) of the magnitude and time to the oxidation of fluorescein (FL) due to peroxy radicals generated by the addition of 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) (22-24). Fluorescein stock solution was prepared with phosphate buffer (25). Trolox standard stock solution were prepared with buffer and used to calibrate standard curves. Plasma samples for ORAC assay (ORAC_{total}) were also diluted directly with buffer. Duplicate blanks, standards, and samples were treated with FL working solution and diluted AAPH, which were added to each solution separately. ORAC_{total} and ORAC_{pca} concentrations were determined on a microplate reader (Fluostar Optima, BMG Labtech, Offenburg, Germany). Intra-assay variation was 3.9%.

Serum samples were analyzed for CRP, IL-6, sICAM-1, sVCAM-1 in duplicate via Enzyme-Linked Immunosorbent Assay (ELISA) using commercially available kits (United Biotech Incorporated, Mountain View, CA). Intra-assay variations were 8.4%, 8.4%, 2.7%, and 2.4%, respectively.

Serum samples were analyzed for FFA, triacylglycerol, and glucose in duplicate via spectrophotometry using a commercially available enzymatic-colorimetric kits (Wako Chemicals USA, Richmond, VA and Stanbio, Boerne, TX), respectively. The adapted microplate procedure was employed for lipid sample analysis for both measures (Wako Chemicals USA, Richmond, VA). Intra-assay variations were 2.6%, 4.1%, and 2.0%, respectively. Insulin was analyzed via radioimmunoassay (Diagnostic Products Corporation (DPC), Los Angeles, CA). Intra-assay variation was 7.1%.

Urine Collection:

Subjects provided first-void urine samples after an overnight 12 hour fast on the mornings of the CRP screening (day 1) and all four meal challenges (days 15, 28, 43, and 56). Urine samples were collected in 100 mL polypropylene containers and were stored at 5°C prior to being aliquoted into cryovials for storage at -80°C until analysis of creatinine and urinary isoprostanes (8-epi PGF_{2α}). Urine samples for isoprostane concentrations were quantified in duplicate via Enzyme-Linked Immunosorbent Assay (ELISA) using a commercially available kit (Neogen Corp., Lexington, KY). Intra-assay variation was 5.0%. Urinary 8-epi PGF_{2α} was expressed per mg creatinine. Urinary creatinine was analyzed in duplicate via quantitative colorimetric determination (Stanbio, Boerne, Texas). Intra-assay variation was 0.7%.

Raisin Analysis:

Raisins were obtained from the California Raisin Marketing Board in multiple batches at the start of the study. All batches were combined, mixed, and aliquoted into 5 large bags and placed in opaque plastic sealable containers and stored in a freezer at 2.5° C until needed. In order to quantify phenolic content of the raisins and to determine whether this changed during storage, 45 gram randomized samples of raisins were taken and frozen at -80°C for later analysis. Samples were collected every 30-60 days prior to each treatment period, during washouts, and at the conclusion of the intervention until all subjects completed the study (total 6 samples over 6 months). For extraction of pigments, a modified procedure was used as outlined by Hong and

Wrolstad (26) and Karadeniz et al. (17), and the total phenolic extractions were analyzed by the Folin-Ciocalteu method by Spanos and Wroltsrad (27). Intra-assay variation was 5.6%.

Statistical Analysis:

Descriptive statistics were used to summarize the data. Baseline student's t-tests were used to identify differences between genders for initial subject characteristics and measures. Repeated measures (RM) ANOVA were used for fasting (times were pre and post) and postprandial (times were 0, 2, 3, 4 h) data. In order to examine postprandial changes in relation to the fasted values for the factor at time 0, each measurement was expressed as a percent change of the initial time 0 value (100%) and a 3 factor mixed model ANOVA was used to determine the main effects of either treatment (raisin or placebo), time (2, 3, 4 hours postprandial), intervention (pre and post), gender (male or female), order (period), and the interaction among these main factors. All analyses were computed via SAS software (version 9.1.3; SAS Institute Inc., Cary, N.C.). A mixed model was used in order to circumvent case-wise deletion of subjects with missing data points. Post hoc analyses were done using the Tukey-Kramer test for significance to identify the source of significant effects. Pearson correlation coefficients were performed for screening blood draw (fasted) actual values (not % change) and measures of obesity; hip to waist ratio, waist circumference and BMI measures as well as metabolite change scores. Change scores were calculated as peak postprandial response less time 0 for all 4 meal challenge. Statistical results for all measures except glucose, sICAM-1, and sVCAM-1 were based on log (ln) or square root-transformation of the percent change data: analyses for CRP, IL-6, ORAC_{total}, ORAC_{pca}, 8-isoPGF_{2α}, and insulin used the log response, the square root response for FFA, and triacylglycerol. Log and square root transformations were done in order to accommodate nonnormal distributions of the data. Insulin RM ANOVA fasted comparisons were based on differences computed by subtracting post from pre treatment values and the significance was based on the difference from 0. A separate analysis was required for insulin since ANOVA assumptions were not upheld under the standard analysis due to a high number of identical values. CI was computed for log-transformed data requiring back transformation in order to obtain group means expressed as actual values. Fasting data was expressed as mean and 95% confidence interval (CI), and data for initial subject characteristics and diet analysis were expressed as mean ± SD unless otherwise stated. A p value of <0.05 was considered significant.

RESULTS:

Subjects:

17 subjects, 8 males and 9 females, ages 26.5 ± 7.6 , successfully completed the investigation. One subject dropped out while two were asked to discontinue participation due to self-report of non-compliance to study requirements relating to treatment consumption.

According to self-reported information in the exit survey, subjects had almost full compliance with daily treatment consumption: 98.7% and 98.8% of doses were consumed for raisin and placebo, respectively.

Throughout the self-selected low flavonoid diet, 59% of subjects (10 subjects) reported consuming foods or beverages from the list of prohibited items. Of the 10 subjects that reported non-compliance, nine ate prohibited foods during the self-selected diet period and 7 ate these foods during the controlled feeding periods. A total of 6 subjects consumed foods and/or beverages during both periods. Types of prohibited foods consumed during the study ranged from a single hamburger to a glass of iced tea or lemonade. No subjects reported regular consumption of the prohibited foods, and occurrences were not considered sufficient to affect results.

During the controlled feeding periods, a total of four subjects had unplanned absences arise ranging from 1 to 3-d. One subject was provided with food and treatment, three were provided with treatment and menus to follow while absent. All absent subjects were required to complete absentee food records when not in attendance.

Diet Records:

According to the 4 day food records, subjects reported consuming an average of 2481 ± 578 kcal per day prior to the start of the study. As indicated by 24 hour recalls and diet records (4-d and from absentee food records) during the experimental period, the average kcal intake was 2059 ± 636 per day. The consumption of vitamin antioxidants (vitamins C, A, and E) were, as intended, considerably reduced during the experimental, low flavonoid and antioxidant dietary periods. Prior to the start of the study, the average antioxidant intakes were 126%, 76%, and 91% the RDA for vitamin C, E and A, respectively⁵. According to 24 hr diet records, subjects reduced their antioxidant intake during the study to 49%, 44%, and 69% of the RDA for vitamin C, E, and A, respectively.

⁵ Percent based on averaged RDA for males and females where appropriate

Weight Maintenance:

As a group, body weight was maintained within an average standard deviation of 0.7 kg and 0.6 kg from initial body weight during the two controlled feeding weeks (d 22-28 and 50-56, respectively). Minor adjustments of 100 kcal carbohydrate snacks were made to stall weight loss when body weight dropped by more than 1-1.5 kg, no adjustment was made for weight gain during the controlled feeding periods. As a group, subjects maintained within an average deviation of 0.9 kg of their initial body weight throughout the entire study as measured on the mornings of the screening blood draw (d-1) and meal challenges (d 15, 28, 43, and 56).

Fasting Blood:

Baseline characteristics and fasting serum analytes for subject groups combined are provided in (Table 2) and initial characteristics by gender (Table 3).

Indices of Initial Health Risk of Subjects:

Subjects with elevated fasting glucose (≥ 100 mg/dL), triacylglycerol (≥ 150 mg/dL) and CRP (>3.0 mg/L high risk) were considered at increased health risk as defined by the American Heart Association and the National Heart, Lung and Blood Institute (5,28). Nearly half, 47%, of the subjects, had serum glucose and CRP less than the cut off for elevated levels prior to meals. Approximately 40% of subjects showed evidence of chronic inflammation with elevated CRP prior to each of the four meals. None of the subjects were hyperglycemic before all four meals, and only one subject was hypertriglyceridemic at each fasted measure (Table 4).

The treatment did not have any significant main effects or interactions with any other factor on fasting levels of CRP, and sICAM-1 (Table 5). There was no effect of treatment but a significant effect of pre/post on IL-6 as shown by an 11% lower IL-6 in post measures. Serum sVCAM-1 exhibited a higher value in post measures (3.7%). Urinary 8-iso $\text{PGF}_{2\alpha}$ was 22% lower in post measures. Fasting serum $\text{ORAC}_{\text{total}}$ was higher in post measures, but was not different by treatment, and fasting serum ORAC_{pca} was higher in post measures by 3.5%. A significant interaction between gender and treatment was demonstrated by a higher ORAC_{pca} for raisins versus placebo in men, the opposite pattern was seen for female subjects (Table 6). FFA measures exhibited a significant interaction between treatment and pre/post, showing a reduction after raisin treatment and an increase after placebo treatment. FFA concentrations were ~9% higher for all raisin measures than placebo, and fasting glucose was ~5% lower after the combined interventions compared to before. Male subjects had higher fasting glucose in raisin

treatment while women had a higher concentration in placebo. There were no significant main effects or interactions on fasting triacylglycerol or insulin.

Postprandial Blood:

Serum CRP, IL-6, sVCAM-1, and sICAM-1 did not significantly change in response to the meal challenge or treatments (Table 6). Interestingly, serum CRP measures exhibited increases in pre measures of +11.8% in response to the meals regardless of treatment, where post treatment was less pronounced at +1.8%. Serum IL-6 showed a downward trend postprandially in all meals combined ($p=0.0546$), and was more pronounced at 2 hrs, with a return to baseline at 4 hrs postprandially. Postprandial serum sVCAM-1 responded differently by gender. Males had a sizeable decrease from baseline at time points 2 and 3 hours (-4.0%) and (-5.1%), where females exhibited little change (Figure 2).

Serum FFA exhibited significant postprandial decreases followed by a gradual increase back to baseline in subsequent measures. The response was more pronounced in the meals that included raisins versus placebo. Genders responded differently, females were at 66% of their baseline measures at hour 4, and males had exceeded their baseline measure by 37.8% (Figure 3). As expected, serum triacylglycerol increased postprandially, however there were no differences between treatments. Gender differences were noteworthy in that males were more likely to have higher and a more prolonged response in postprandial triacylglycerol concentrations than females.

Serum glucose increased postprandially with a more pronounced increase with placebo versus treatment. Gender responses differed in that males showed an increase of +10.4% for males and +1.2% for females above baseline, with males exhibiting a more pronounced and prolonged response to the meals when compared to females (Figure 4).

Serum insulin rose postprandially in response to the meals, peaking at time 2 h (+248.8%), and decreased at 3 and 4 h (147.3% and 70.9%). There were no other significant main effects or interactions in post hoc analyses, and no differences between treatments or gender.

Raisin Analysis:

Of the 3 raisin samples analyzed, the total phenolic compounds extracted were 755, 828, and 1050 gallic acid equivalents/kg raisins. The average intra-assay coefficient of variation was 5.6%.

DISCUSSION:

The intent of this study was to investigate the value of raisins as a means to reduce oxidative stress, endothelial dysfunction, and inflammation in overweight and obese subjects. However, while the raisin treatment did not have expected effects on inflammation or oxidative stress, the controlled feeding (whether with daily raisin or placebo ingestion) decreased oxidative stress (lower urinary 8-isoPGF_{2α}), enhanced the subjects' potential to lower oxidative stress (higher ORAC), exhibited lower levels of inflammatory mediators (IL-6), and reduced glycemia. Eating in a regular pattern without skipping meals, binge eating or dining out (Western-style dietary pattern), and overall nutritional composition of the meals may have improved these characteristics in our healthy, but overweight and obese population (29-31).

An additional illustration of the effects of meal patterns and health outcomes can be seen via the current dietary practices utilized in medical nutrition therapy for diabetes education. Regular meal patterns consisting of 3-6 regularly spaced meals per/day with portion control has been shown to lower the proportion hb_{A1c} in diabetic populations, demonstrating improved glycemic control (32). In our study, subject's pre-intervention food records demonstrated a western-style meal pattern. Our controlled feeding period meals were modeled after AHA macronutrient recommendations (55% CHO, 30% fat, and 15% protein), in order to eliminate the effect of macronutrient profile and to provide regularly spaced meals. These findings support the importance of separating the effects of a controlled feeding from that of the treatment in future studies.

Outside of dietary patterns, effects of raisin treatment were mainly limited to the improvement of the subjects overall potential to reduce oxidative stress. This was illustrated by an increase in fasting measures for ORAC. ORAC is a measure of the overall radical scavenging ability of water soluble antioxidants in a biological sample. It is a beneficial measure for biological samples because of its ability to quantitate a complex array of antioxidants with different properties. The primary water soluble antioxidants in serum include uric acid, vitamin C, various phenolic compounds, caffeic and chlorogenic acid, catecholestrogens, ferulic acid, Trolox C (water soluble form of vitamin E), and aminoguanidine (33). Dietary consumption of antioxidants plays a major role in serum concentrations of these compounds, as well as influencing innate antioxidant enzymatic pathways (11).

Investigators utilizing grape products have shown increases in ORAC and flavonoid concentrations (11,20,34). O'Byrne et al. showed an increase in ORAC after 2 weeks of daily Concord grape juice intervention. Caccetta et al. found an increase in serum flavonoids following a wine intervention with no significant effect on LDL susceptibility to oxidation. Subjects served as their own controls and consumed their regular *ad libitum* diet during the study (34). Our findings showed an increase in serum ORAC in post controlled feeding measures. These effects were seen regardless of treatment. Similar to our findings, Dragsted et al. (35) found no effect of increasing fruit and vegetable intake for 7 d on the free radical scavenging ability of plasma compared to a placebo treatment. Unfortunately, it is beyond the scope of this study to determine if any of the dietary components actually increased in vivo or if less oxidants were produced as a result of a regular meal pattern.

With the increase in serum ORAC one would expect a concurrent decrease in our measure of oxidative stress, urinary 8-isoPGF_{2α}. This measure was not influenced by our treatment, but was seen to decrease by 22% after intervention periods in the study. Other studies have found similar conflicting results (36). Edgar et al. found no difference in their measure of oxidative stress (malondialdehyde – MDA) after 11 weeks of a daily serving of 9-10 fruits and vegetables to 123 weight stable healthy adults. Edgar's group found non-significant increases in ORAC in both of their treatments (fruit and vegetables, fruit vegetables and low fat), but not in their control group (36). Conversely, Zern et al. found a significant reduction in urinary 8-epi in pre and post menopausal women after supplementation with grape extract (10).

The overall health of a population may influence the effect of the meal stressor and/or treatment. Our study population may have been too healthy, diminishing meal responses and/or treatment effects. Other studies utilizing less healthy subjects, diabetics or smokers, have shown stronger responses (7,37). Nappo et al. investigated diabetic and healthy subject's response to high fat and high CHO meals. Diabetic subjects had higher baseline serum levels of glucose, VCAM-1, ICAM-1, and TNF_α, and exhibited significant amplified responses to both meals compared to healthy subjects (7). Unlike our study, Nappo's group showed an improvement in inflammatory and endothelial responses with antioxidant supplementation in healthy subjects (7). To better understand the complexity of the innate antioxidant and inflammatory systems and their relationships with dietary sources of nutrients warrant future studies that incorporate multiple measures for oxidative stress.

Our findings did illustrate some interesting gender differences. Postprandial measures showed men had greater increases and more prolonged response to meals (FFA, sVCAM-1, glucose and triacylglycerol), when compared to females. This same effect has been seen in other studies (38). Conversely, other investigators noted no differences between genders (39). Abbasi et al. induced a physiological state of induced hyperinsulinemia in 22 healthy, non-diabetic adults. The more insulin-resistant subjects were found to have significantly higher fasting levels of glucose and FFA with no differences between genders (39). Traditionally males tend to present with abdominal obesity more so than females. As Halkes et al. found waist circumference to be significantly associated with elevated postprandial lipemia, this would be expected.

The dietary source of antioxidants is also a factor that plays a role in the bioavailability of an antioxidant. A good number of studies have used concentrated extract sources of flavonoids and phenolic compounds, such as grape products, grape juice, and wine (10,14,40,41). When using whole foods, there are other constituents that influence the digestion and absorption of all individual food compounds: fiber and other polymers, pH, and competitive transport. One might assume that whole raisins and grapes would be similar to these extracts and that grapes would have the same nutrient value of raisins, their dried counterparts. However when raisins undergo the drying process, there are losses of some flavonoids as well as some polymerization of phenolic compounds that reduce their bioavailability (17). According to Karadeniz et al., raisins and grapes contain various phenolic and flavonols. Of the phenolic compounds (caftaric and coumaric acids), raisin concentrations decreased as a result of drying from 103.4 to 39.6 mg/kg and 39.8 to 6.7 mg/kg of sample, respectively. Oxidized cinnamates increased in raisins and were not present in fresh grapes. Of the flavonols, rutin, quercetin glycosides, and kaempferol glycosides, rutin increased from 0.9 to 5.7 mg/kg, as well as the glycosides combined, from 45.2 to 76.9 mg/kg of sample. Finally, Arts et al. found concentrations of flavon-3-ols, catechin and epicatechin in raisins, where Karadeniz's group did not analyze these compounds (18). It is probable that the dosage of raisins was not sufficient to alter measures of inflammation due to this known decrease in bioactive compounds. The raisin dosage used in our study was chosen based on two additional fruit servings a day, a realistic quantity that people would likely consume. According to our analyses, the phenolic composition of our intervention ranged from 68-94.5 mg/d of phenolics (mg gallic acid equivalents). In contrast to our findings, Karakaya

group's analysis of raisins produced 359.5 mg total phenolic compounds per 90 grams (43). However, our phenolic dose was lower than most values in grape, grape product and grape juice studies which used a range of 300-560 mg/dl (10,20,42, 43).

We believe that the duration of the study was adequate to show effects of the intervention. Similar studies using grape products utilized 14-15 day periods in their investigations (20,29,41). These studies exhibited positive effects by decreases in oxidative stress markers (20), and/or increases in antioxidant potential of serum (20). One major limitation is the use of free-living subjects in our study. Free-living subjects bring additional factors that may influence dependent variables and are impossible to account for.

SUMMARY AND CONCLUSIONS:

In summary, fasting measures for ORAC were increased in both raisin and placebo interventions, more so with raisins. Consuming raisins each day did not affect markers of oxidative stress, inflammation, or endothelial activation following a high fat meal. Finally, it's possible that a controlled regular meal pattern influenced markers of oxidative stress and mediators of inflammation regardless of treatment. Further investigation would benefit from methods that would help dissociate meal patterns from dietary interventions.

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Table 3.1. 2500 kcal Day: Sample Menu from Controlled Feeding

Meal	Food
Breakfast	1.5 scrambled egg
	1 biscuit (60 grams)
	226.8g canned pears
	21.26g jelly
	45g raisins
Snack	4 peanut butter crackers
Lunch	357g chicken noodle soup
	8 saltine crackers
	1.5 grilled cheese sandwich: 3 slices white bread (50g), 1 slice American cheese (24.26g)
Dinner	cheese burger: 5 oz ground beef patty (70% lean), 1.5 (64.5g) hamburger bun, 24.26g American cheese slice, 9.17g mayonnaise, 23.44g yellow mustard, 24g iceberg lettuce
	45g raisins
Snack	56g pretzels
	2 puddings (116g each)

3-day rotating menus were utilized during the 7 d controlled feeding period. Diets were targeted to meet 1700, 2000, 2500, 3000, 3500 kcal/day requirements for weight maintenance. All menus were analyzed for nutrient composition prior to the commencement of the investigation (Nutrition Data System for Research (NDS-R version 5.0_35), Nutrition Coordinating Center, University of Minnesota, Minneapolis, MS)

Table 3.2. Initial Characteristics of the Study Population

n	17
Age (years)¹	26.5 ± 7.6
Gender (M/F)	8/9
Weight (kg)	97.2 ± 19.8
BMI (kg/m²)	33.5 ± 6.7
Waist Circumference (cm)	107.4 ± 12.7
Waist to Hip Ratio (WHR)	0.91 ± 0.08
Free Fatty Acids (μmol/L)	345 ± 189
Triacylglycerol (mg/dL)	125.8 ± 100.1
Glucose (mg/dL)	97.4 ± 11.8
Insulin (μIU/ml)	13.2 ± 10.5
CRP (mg/L)	5.5 ± 5.8
IL-6 (pg/mL)	1.5 ± 1.1
sICAM-1 (ng/mL)	224.5 ± 50.7
sVCAM-1 (ng/mL)	695.8 ± 136.8
ORAC_{total} (μmol/L TE)	8392 ± 1273.6
ORAC_{pca} (μmol/L TE)	1011.8 ± 163.4
Urinary 8-epi PGF_{2a} (pg/mg Cr)[‡]	4271.9 ± 1203.7

Values are mean ± SD. Values before the start of a low-flavonoid diet. [‡]CR, creatinine

Table 3.3. Initial Characteristics of the Study Population by Gender

	Males	Females
n	8	9
<i>Age (years)</i>	30.1 ± 7.3	23.2 (6.6)
<i>Weight (kg)</i>	100.4 ± 17.5	94.3 ± 22.2
<i>BMI (kg/m²)</i>	31.4 ± 5.1	35.3 ± 7.5
<i>Waist Circumference (cm)</i>	106.8 ± 11.4	108.0 ± 14.5
<i>Waist to Hip Ratio (WHR)</i>	0.95 ± 0.10	0.87 ± 0.03
<i>Free Fatty Acids (μmol/L)</i>	331 ± 154	355 ± 214
<i>Triacylglycerol (mg/dL)</i>	159.1 ± 131.9	96.2 ± 52.2
<i>Glucose (mg/dL)</i>	95.6 ± 12.7	98.9 ± 11.5
<i>Insulin (μIU/mL)[†]</i>	12.5 ± 11.4	18.1 ± 12.4
<i>CRP (mg/L)</i>	2.8 ± 4.2	8.3 ± 6.2
<i>IL-6 (pg/mL)</i>	1.2 ± 1.2	1.7 ± 1.1
<i>sICAM-1 (ng/mL)</i>	206.8 ± 49.6	240.3 ± 48.9
<i>sVCAM-1 (ng/mL)</i>	717.6 ± 140.4	676.3 ± 138.9
<i>ORAC_{total} (μmol/L TE)</i>	8192 ± 1118	8570 ± 1441
<i>ORAC_{pca} (μmol/L TE)</i>	1033 ± 204	993 ± 127
<i>Urinary 8-epi PGF_{2α} (pg/mg Cr)[*]</i>	4101.9 ± 1172.1	4423.0 ± 1281.0

Values are mean ± SD. Values before the start of a low-flavonoid diet. [†]Significant difference between genders (p<0.04). *CR, creatinine

Table 3.4. Frequency of Elevated Indices of Health Risk

Percent of Subjects (Total 4 Meals)	Never	1 Meal	2 Meals	3 Meals	4 Meals
<i>Glucose ≥ 100 mg/dL</i>	47% (n=8)	35% (n=6)	12% (n=2)	6% (n=1) ¹	0% (n=0)
<i>Triacylglycerol ≥ 150 mg/dL</i>	59% (n=10)	23% (n=4)	12% (n=2)	0% (n=0)	6% (n=1) ¹
<i>CRP >3.0 mg/L (high risk)</i>	47% (n=8)	6% (n=1)	6% (n=1)	0% (n=0)	41% (n=7) ¹

¹ Same subject. All data was based on fasting levels (time 0) prior to consuming the meals. Percent of subjects was based on n/17 that had qualifying values for the marker of interest.

Table 3.5. Fasting Values for Pre and Post Interventions

Analyte	Pre Raisin	Post Raisin	Pre Placebo	Post Placebo
CRP (mg/L)	2.18 (1.03-4.66)	2.24 (1.05-4.80)	2.20 (1.03-4.70)	2.15 (1.01-4.58)
IL-6 (pg/mL)¹	1.23 (0.78-1.78)	1.08 (0.66-1.60)	1.23 (0.78-1.78)	1.02 (0.61-1.52)
sVCAM-1 (ng/mL)¹	657.94 (585.76-730.12)	698.98 (626.80-771.16)	671.88 (599.70-744.06)	678.93 (606.75-751.12)
sICAM-1 (ng/mL)	208.278 (189.113-228.368)	209.996 (190.750-230.167)	209.599 (190.371-229.751)	199.245 (180.510-218.904)
ORAC_{total} (μmol/L TE)^{1,2}	7163.03 (6479.27-7918.93)	7613.95 (6887.15-8417.44)	8335.73 (7540.04-9215.39)	8403.14 (7601.01-9289.92)
ORAC_{pca} (μmol/L TE)^{1,2}	886.03 (822.86-954.10)	932.32 (865.82-1003.92)	980.07 (910.16-1055.34)	1014.76 (942.38-1092.69)
Urinary 8-epi PGF_{2α} (pg/mg Cr)^{*1}	4164.06 (3538.31-4900.47)	3107.58 (2640.59-3657.16)	4298.21 (3665.29-5040.43)	3484.95 (2971.78-4086.73)
FFA (μmol/L)^{1,2}	371 (309-439)	346 (286-412)	256 (205-314)	338 (278-403)
Triacylglycerol (mg/dL)	91.0 (66.8-123.9)	92.7 (68.0-126.2)	99.1 (72.8-135.1)	91.8 (67.4-125.0)
Glucose (mg/dL)¹	94.8 (91.4-98.3)	88.8 (85.5-92.2)	92.5 (89.1-95.9)	88.8 (85.5-92.1)
Insulin μIU/mL	12.5 ± 10.5	9.4 ± 6.5	10.5 ± 8.5	10.4 ± 9.0

Values are means with 95% CI for time 0 blood draws prior to meals. *CR, creatinine.

¹Significant effect of pre to post interventions combined.

²Significant difference between raisin and placebo

Table 3.6. Postprandial Changes: Raisins and Placebo

Analyte	Treatment	2 hrs	3 hrs	4 hrs
CRP (mg/L)	P	16.8 (79.51)	66.9 (288.74)	55.9 (272.90)
	R	23.04 (76.67)	72.2 (294.93)	65.7 (257.02)
IL-6 (pg/mL)	P	-15.9 (35.2)	-1.7 (56.6)	36.7 (170.6)
	R	-25.8 (38.8)	-5.6 (65.4)	-14.5 (25.4)
sVCAM-1 (ng/mL)⁴	P	-1.5 (5.9)	-0.4 (7.15)	-0.04 (7.04)
	R	-1.8 (6.51)	-3.7 (8.01)	-3.2 (7.51)
sICAM-1 (ng/mL)	P	-1.3 (5.4)	-1.3 (8.8)	-1.2(9.1)
	R	-1.0 (11.0)	-2.3 (14.7)	0.8 (13.5)
FFA (μmol/L)^{1,2,3,4}	P	-58.4 (23.96)	-32.03 (35.07)	-6.8 (39.40)
	R	-54.2 (43.30)	-12.6 (71.60)	-21.1 (84.15)
Triacylglycerol (mg/dL)	P	35.1 (34.9)	66.7 (57.3)	74.5 (72.9)
	R	50.3 (39.7)	77.4 (50.9)	94.9 (52.8)
Glucose (mg/dL)^{1,3,4}	P	2.5 (15.6)	3.0 (10.10)	6.0 (12.41)
	R	4.7 (15.34)	7.8 (13.39)	10.9 (12.91)
Insulin μIU/mL¹	P	289.9 (217.5)	173.8 (162.0)	80.0 (91.7)
	R	310.8 (209.8)	193.2 (146.1)	106.2 (99.3)

Values are percentage change from time 0 with SD in parenthesis

R, Raisin treatment, P, Placebo treatment

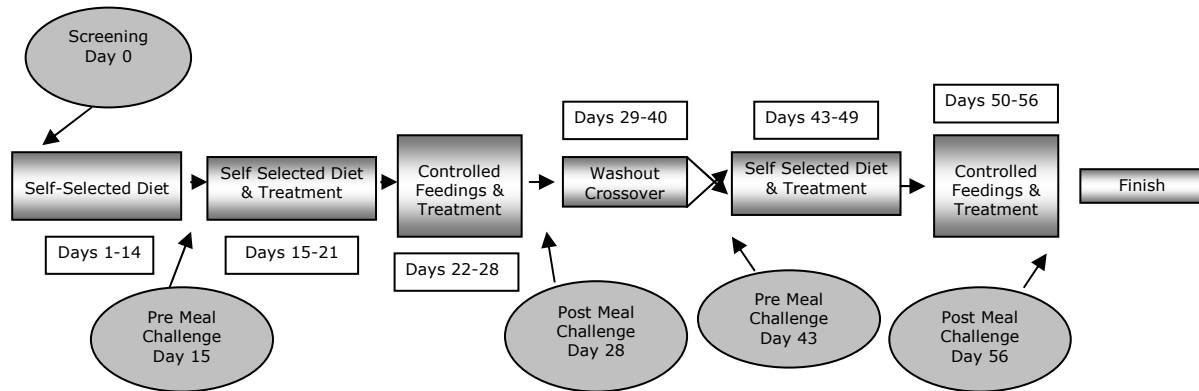
¹Significant effect of time.

²Significant effect of treatment.

³Significant effect of gender.

⁴Significant interaction between gender and time.

Figure 3.1. Study Design



The study model was a randomized, cross-over design. The duration of the study for each subject totaled 56 days: Days 1-14 consisted of a lead-in low-flavonoid diet, followed by 14 days (15-28) of low flavonoid diet plus the first intervention. Subjects were provided with all their food by investigators during days 22-28, and 50-56. The crossover consisted of 14 days (29-42) of washout between interventions. The study was completed by the final 14 days (43-56) of the alternate intervention

Figure 3.2. Interaction of Gender with Time for Postprandial Soluble Vascular Cellular Adhesion Molecule-1

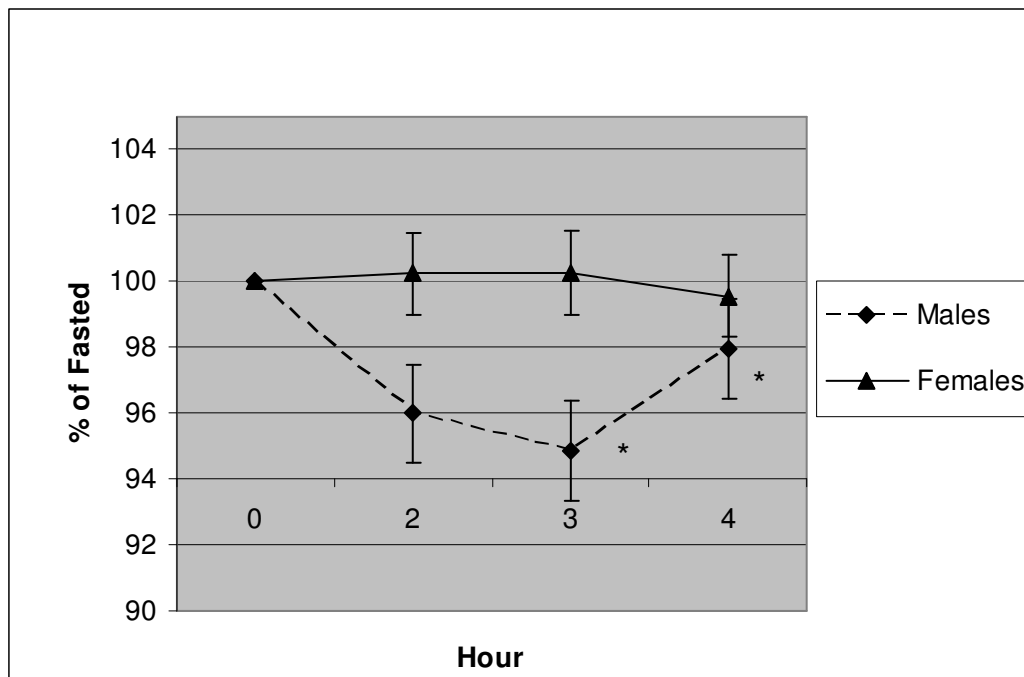


Figure 3.3. Interaction between Gender and Time for Postprandial Serum Free Fatty Acids

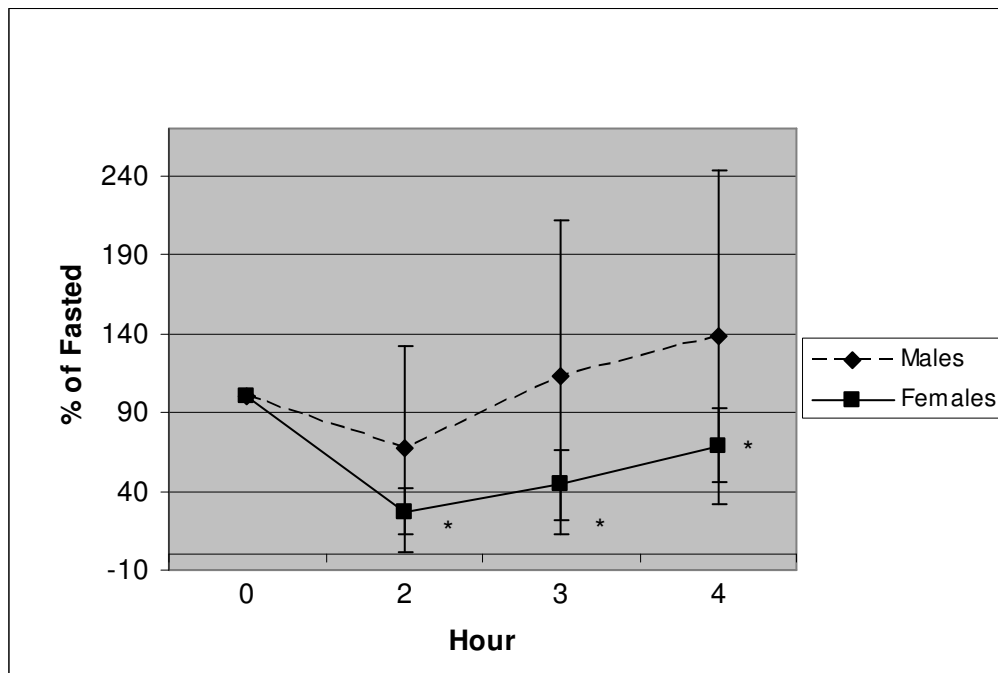
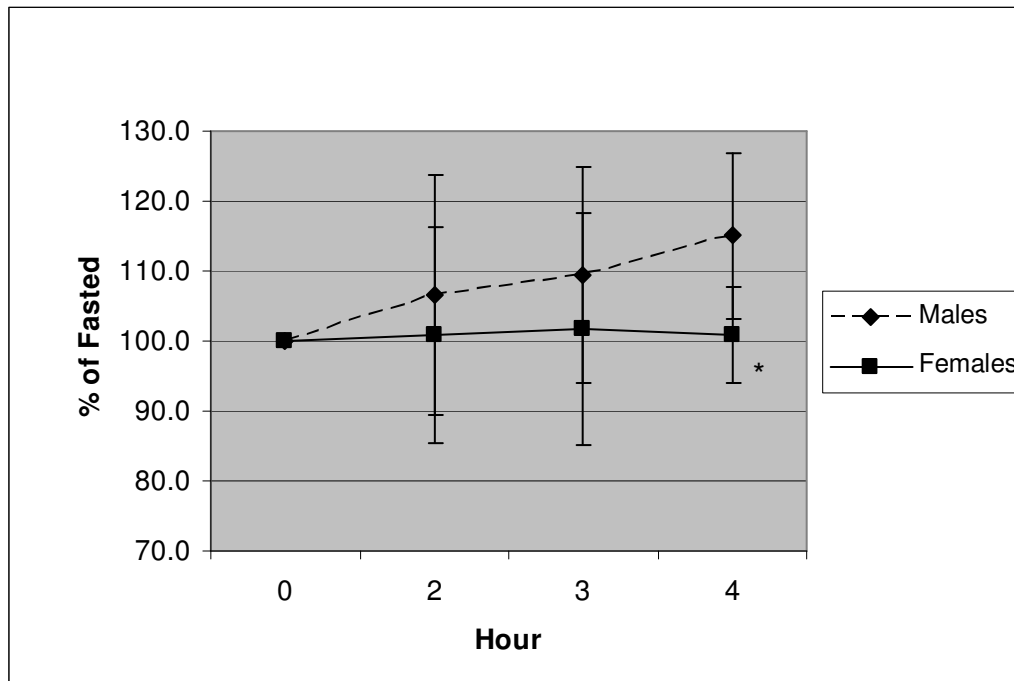


Figure 3.4. Interaction between Gender and Time for Postprandial Serum Glucose



CHAPTER 4:
Summary and Recommendations

The incidence of overweight and obese individuals worldwide is increasing and contributing to the risk for chronic diseases such as CVD. Lifestyle changes that cause weight loss can reduce this risk; however weight loss and maintenance are difficult for most people to achieve. Thus, clinicians are interested in lifestyle strategies that can reduce chronic disease risk without weight loss.

One link between chronic disease and adiposity is the chronically elevated inflammatory and oxidative stress status in the overweight and obese. Elevated inflammatory markers such as CRP, IL-6, and TNF- α , and oxidative stress markers such as urinary 8-iso PGF_{2 α} have been associated with increased incidence of endothelial activation and dysfunction (demonstrated by elevated sICAM-1 and sVCAM-1), and higher risk of vascular events. Endothelial dysfunction is believed to occur when inflammatory and oxidative stress processes interfere with NO bioavailability depressing vasodilatory capabilities. If the endothelium becomes less flexible, damage to the tissue results in macrophage infiltration, fatty streak and plaque formation. Unstable plaques are an outcome of uninhibited inflammation. Plaque rupture results in thrombus formation and vascular events such as myocardial infarction and stroke.

Some studies observe that individuals with obesity have a magnified postprandial response with increased insulin resistance (high serum insulin), elevated glucose, TAG and FFA. All of these factors are individually linked to elevated markers of inflammation, oxidative stress and endothelial dysfunction. For example, Studies have demonstrated that high fat meals acutely increase inflammatory or oxidative stress markers, tumor necrosis factor- α (TNF- α), IL-6, CRP, F₂-isoprostanes (8-iso prostaglandin F_{2 α} (8-iso PGF_{2 α})), sICAM-1, and sVCAM-1 (8). As much of each day is spent in the postprandial state, repeated elevation of these factors could play a key role in elevating CVD risk in this population (8).

The addition of antioxidants vitamins E and C, flavonoids, and adherence to the Mediterranean diet, have been shown to ameliorate some of the negative postprandial effects, suggesting that this response is linked to the ability of the body to quench free radicals (10). This suggests that inclusion of foods with substantial antioxidants may attenuate the postprandial inflammatory response.

Fruits and raisins are known to have a variety of polyphenolic constituents such as flavonoids, phenolic acids and tannins (27). Epidemiological studies looking at overall vegetable, fruit, wine and other grape product consumption demonstrate an association with lower CVD

(15), suggesting that flavonoids or other compounds found in these foods are responsible for the effect on disease risk. Although there is evidence that a flavonoid-rich diet is associated with reduced health risk and CVD (19), it is still unclear whether or not this is due to a reduction in oxidative stress and/or inflammatory state. Some investigations have explored the effects of total fruit and vegetable intake on markers of oxidative stress, showing the reduction in several markers (14). Several investigations have found positive outcomes when looking at the effects of flavonoid rich foods, oxidative mechanisms, as well as vasodilatory capabilities (22, 23). Wines, grapes, and raisins are whole foods that are high in flavonoids (27). Studies showing health benefits of wine and grape juice suggest that raisins may have similar benefits as their non-dried counterparts (19, 34). To the best of our understanding, no other interventions have used raisins as a whole food intervention in the overweight or obese.

It was of interest to us to determine whether the addition of foods containing antioxidant compounds, such as raisins, to the daily diet of overweight and obese individuals could reduce their basal as well as postprandial inflammatory state, and thus reduce oxidative stress and endothelial dysfunction.

An unexpected outcome of our study was the minimal changes in inflammatory or endothelial markers that occurred in response to the high fat meal. Thus, it was difficult to determine whether any intervention improved postprandial response. Nevertheless we observed that the controlled feeding period (whether with daily raisin or placebo ingestion) decreased oxidative stress (lower urinary 8-isoPGF_{2α}), and enhanced the subjects' potential to lower oxidative stress (higher ORAC). Subjects also exhibited significantly lower levels of fasting inflammatory mediators (IL-6), and reduced fasting glycemia.

The lack of response to raisin treatment could be attributed to a number of factors, such as raisin versus grape composition. Other studies have utilized grape extracts, juice and wine. We know that raisin flavonoid composition differs from raw grapes and grape products used in these other studies (27). For example, raisins contain no resveratrol, one flavonoid of interest in red wine. Resveratrol is found primarily in the skin of grapes. As an antioxidant, resveratrol's main CVD related effects include decreasing LDL oxidation susceptibility in vitro and supporting endothelial NO release (110). The reduction in raisin flavonoid content, particularly resveratrol, results primarily from the drying process, as shown by Karendiz et al. (27). Also, raisin flavonoid content may have additional decreased bioavailability due to polymerization

when dried (111). Therefore, comparable dose requirements for measurable health benefits may have exceeded the 90 grams of raisins (concentration of flavonoids) given in our study. According to Karakaya et al., raisins contain 359.5 mg total phenols per 90g serving compared to 142.2 mg for green and 198.5 mg for red grapes (also 90 gram servings) (32). Our two 45 gram doses (90g) should have provided a similar amount of phenolic compounds. Based on these values, our phenolic dose was comparable to most amounts used in other studies using grape, grape product and grape juice (range of 300-560 mg/dl) (8, 18, 33). According to our method of flavonoid quantification (Folin-Ciocalteu) our highest concentration (94.5 gallic equivalents/90 grams raisins) was only 27% of what Karakaya's group reported. Puglisi et al looked at the effects of raisin (1 cup/d) consumption for 6 weeks with or without a walking intervention on CVD markers, sICAM-1, LDL-C, TAG, and TNF- α (112). All groups had decreases in LDL-C, TAG, and sICAM-1. The raisin group saw a significant decrease in the inflammatory marker, TNF- α . These findings indicate that our dose of raisins may have been inadequate as well as the duration of our intervention. Bioavailability may very well be a contributing factor, requiring larger doses for longer periods of time for the desired effects.

It is known that there is a strong correlation between increased urinary 8-iso PGF_{2 α} and obese populations, making this an appropriate measure of oxidative stress for our purposes. Alternative measures used in flavonoid studies include *ex vivo* LDL susceptibility to oxidation, another measure of oxidative stress or lipid peroxidation (93, 113). In some investigations, both measures have been employed. For example, Zern et al. (19) showed decreases in urinary 8-isoPGF_{2 α} in overweight and obese women after supplementation with grape extract. However, Zern's group saw no change in LDL susceptibility to oxidation after their treatment (19). However, LDL oxidation measures may be more relevant in flavonoid studies using healthy subjects. O'Byrne et al. (34) found a decreased lag-time for LDL oxidation after healthy adults were supplemented with Concord Grape Juice. The use of this measure as well as serum concentrations of flavonoids may aid in separating the effects of the controlled feedings and treatment.

Despite unexpected outcomes regarding a value of raisins, our most noteworthy findings were a 20% decrease in our oxidative stress marker, fasting urinary 8-isoPGF_{2 α} , as well as a significant increase in fasting ORAC. Other investigators had similar findings (17), and attributed this to the regular meal pattern of the controlled feeding periods in their study design.

The typical western diet consists of irregular meal patterns, usually including bingeing and meal skipping. Therefore, providing adequate energy to maintain weight, and evenly distributing calories between 3 meals and snacks may have an additional benefit to eating a high antioxidant/flavonoid diet.

In our study, fasting measures such as the inflammatory markers CRP and IL-6 did not change significantly. However, IL-6 decreased 11% from pre to post regardless of treatment. This may have been a residual of the controlled feeding period as well, and may have been related to the decrease in our oxidative stress measure. Other studies have shown similar decreases in oxidative stress markers and inflammatory markers (65). Endothelial markers, sICAM-1 did not significantly change, yet sVCAM-1 did have a significant change from pre to post with a 3.7% increase in post measures. Other investigators have found differences in the expression of adhesion molecules, but in the postprandial state. Chiu et al. investigated the effects of sheer stress on endothelial cells, finding a TNF- α in induced both mRNA and proteins for sICAM-1 while decreasing sVCAM-1 expression (114). Sheer stress, or blood pressure was not a focus of our study, however these findings illustrate that different environmental conditions in the vasculature can have diverse outcomes in endothelial activation. Epidemiological studies have also shown a higher risk of cardiovascular events with elevated sICAM-1 but not with elevated levels of sVCAM-1 (6). We know that elevated levels of glucose and TAG are associated with endothelial activation (8, 115). We found there were no noteworthy changes in fasting metabolites or hormones in our study, most likely due to the overall good health of our subjects.

Postprandial endothelial markers and metabolites did not change significantly, indicating that the endothelium was not seriously activated by postprandial changes in metabolites. Again we attributed this to the good health of our subjects. As stated before, healthy subjects tend to have less of a robust postprandial response (8). The majority of our subjects were normoglycemic and insulinemic at baseline, thus decreasing the likelihood of a more robust response to a high-fat meal. Investigators have shown that postprandial hyperglycemia and hypertriglyceridemia have a cumulative effect on increases in sICAM-1 and sVCAM-1 expression in both diabetic and normal subjects (90). Ceriello et al. used a 1000 kcal combination high-fat and high glucose meal challenge to elicit postprandial endothelial responses: meal composition was 75g fat and 75g glucose (heavy cream and glucose) (90). Our

meal challenge was composed of whole foods, amounting to 700 kcal, 53g fat, and 75-79g carbohydrate (including raisins or placebo). The difference in energy, primarily from fat, as well as whole foods versus a liquid meal, may explain the lack of response to our meal challenge. Like our findings, other investigators have shown similar postprandial endothelial responses in healthy subjects (8). Nappo et al. saw very little change in sICAM-1 and sVCAM-1 in healthy subjects after high carbohydrate meal, where there was a greater response to high fat (8).

We found serum sVCAM-1 decreased more notably in males after the meals when compared to females. Nappo et al. found a decrease in sVCAM-1 at four hrs after a high carbohydrate mixed meal in healthy subjects (8). However no gender differences were noted. In our study, males also exhibited a more prolonged elevated response in serum TAG and glucose. These findings may be attributed to the males presenting with a more characteristic abdominal adiposity distribution when compared to females. Males also had a more muted postprandial FFA decline, with a rebound 38.7% above fasting levels at 4 hours. These findings support others studies, in that visceral obesity is correlated with elevated blood glucose and triacylglycerol (115). Our results did not find a significant relationship between genders and inflammatory or oxidative stress markers. Again, this may be attributed to the general good health of our subjects. Other investigators have found stronger relationships between gender, visceral adiposity and CVD risk in subjects with known disease, such as, diabetes, smokers, and those with CVD (115-117).

In postprandial inflammatory markers, CRP and IL-6 did not significantly change; therefore our meal challenge did not induce a measurable inflammatory response. Other studies using high-fat meal challenges had similar responses in healthy insulin-sensitive subjects, where there was little change seen in CRP and TNF- α (8, 115). However, increases in postprandial IL-6 were reported (8, 115). In our investigation, IL-6 trended ($p=0.0546$) down after all meals despite treatment. This effect cannot be explained. It's possible that a postprandial increase in blood volume may dilute concentrations of IL-6, however this would have impacted other measures as well. Interestingly, postprandial CRP did increase insignificantly by 11% at pre measures and only 1.8% after post measures. The changes in postprandial CRP may be related to decreases in fasting measures of IL-6, which decreased by 11% from pre to post measures.

In summary, our findings did not support the use of raisins as a whole food antioxidant in overweight and obese individuals. However, incorporating raisins as a part of high flavonoid diet

may be beneficial in reducing overall CVD risk in this population. Eating meals in a regular meal pattern may also be beneficial in reducing oxidative stress, increasing the body's ability to quench free radicals, and possibly improving glycemia.

Ideas for future research:

- (1) Our study may not have used a high enough dose of flavonoids to elicit our desired effects. Increasing the dose of raisins to more comparable level of flavonoids may be beneficial. As another study has done (112), using 1 cup of raisins may provide enough flavonoids for more desirable outcomes.
- (2) Thompson seedless raisins (sun dried) contain lower concentrations of phenolic compounds compared to other types, such as golden raisins (dipped) (118). Studies using golden dipped raisins as an intervention may see a more robust response to treatment.
- (3) Analysis by HPLC of the flavonoid content in the raisins would have been beneficial in identifying known compounds and their concentrations in comparison to prepared standards. In our study we used the Folin-Ciocalteu (119) method. This method is used to quantitate the total phenolic content of a natural product. The mechanism employed is an oxidation/reduction reaction. Various compounds may interfere with this method by either increasing or decreasing values. These include: sugars, iron, vitamin C; all of which are found in raisins.
- (4) We did not have a separate control arm with an omitted controlled feeding portion in our study. A study design that uses another group of subjects to undergo the same treatments and serve as their own controls (similar to our study) but with no controlled feeding period would help separate some of the confounding variables found in our investigation.
- (5) A study design that would allow subjects to consume foods from their habitual diet but in a regular pattern may be interesting in order to tease out the effect of a preplanned menu.

- (6) We did not recruit subjects with known disease. Studies have shown a more robust response in these populations. Since our findings can only be applied to healthy subjects ages 18-50, Caucasian and African American, and we cannot conclude that same could be said for those with disease. Raisins may be more beneficial in populations with more oxidative stress and inflammation. Since healthy subjects may not have as much room for improvement in such measures, less healthy people may reap more measurable benefits.
- (7) In our study, our subjects were free living, and controlled feedings were conducted in a learning food lab on campus. For the tightest control on potentially confounding effects of other foods consumed or activity, all subjects would be required to stay in a facility with an adjoining metabolic food lab in order to tightly control subject nutrient intake. Studies that utilize this type of method for controlled feedings are less likely to question diet compliance.

Appendix A

Comprehensive Description of Research Methods and Procedures

SUBJECT RECRUITMENT AND QUALIFYING SCREENING:

Twenty overweight and obese (BMI 26-45) men and women ages 18 to 40 years old were recruited for this investigation. Seventeen subjects completed the study; one subject dropped out and two subjects were asked to discontinue participation due to non-compliance with the study protocol. Subjects were free of any known or diagnosed inflammatory conditions including cardiovascular disease, arthritis, diabetes, hypertension, or smoking. In addition, subjects were excluded if they confirmed weight fluctuations (± 0.5 kg) in the 3 month prior to the study, and exercise in excess of 120 minutes per week (moderate to intense activity).

The study required two separate waves of subjects: Six participated in the first wave (March-April), and eleven subjects participated in the second wave (June-July). Recruiting methods for both waves were identical. The first wave was recruited in February, and the second wave was recruited in April-May.

Tools used for recruitment included; fliers on and off campus, printed posters on the Blacksburg Transit, listserv email to undergraduate, graduate, and faculty and staff at Virginia Tech, word of mouth, and subject incentive (see Appendix K). Subject incentive was defined as a twenty dollar stipend awarded to individuals who helped recruit project participants, the provision being that the participants needed to complete the study. Subjects responded to recruiting tools via an email set up for the purpose of corresponding with subjects in this investigation.

Informational meetings were conducted in order to inform potential subjects on the experimental design. Information included an outline of the study design, such as controlled feeding periods, as well as meal challenge and blood draw days (see Appendix H). A list of foods provided during the controlled feedings and meal challenge days was made available in order to establish any potential food aversions or allergies that would prevent participation. Information on the low-flavonoid diet was provided as well. In addition, interested subjects were instructed on how to properly complete a 4-day food record (3 weekdays, 1 weekend day) to be completed prior to the initiation of the low-flavonoid diet. Instructional handouts accompanied the food record for assistance, as well as the use of food models for portion size estimation. Food records were collected in order to estimate subjects' habitual macronutrient profile as well as micronutrient intake. Of particular interest were micronutrients with known antioxidant properties such as vitamin E and C. Subjects were reminded to cease any nutritional supplements

or anti-inflammatory medications at this time (when applicable). Subjects reviewed and signed informed consent forms as outlined by the IRB (see Appendix G).

All food records were collected on the day 1 of the study. Food records were analyzed via Nutrition Data System for Research (NDS-R version 5.0_35), Nutrition Coordinating Center, University of Minnesota, Minneapolis, MS.

COMPREHENSIVE BASELINE: BLOOD DRAWS AND ANTHROPOMETRIC MEASURES

Subjects were scheduled for a CRP screening blood draw two weeks prior to the first meal challenge. Subjects began the eight week low-flavonoid diet at the time of their screening (the lead-in diet). Subjects arrived at the lab at 7AM after a 12 hr overnight fast. Weight in kg was measured on a digital scale (ScaleTronix, Wheaton, IL), height in cm (Detecto Scale, Webbcity, MO), as well as hip and waist circumference measurements in cm. Waist circumference was measured with a plastic tape meter at the level of umbilicus or narrowest point of the waist, and at the widest point across the gluteus for the hip . Subjects with CRP levels greater than 20 mg/L were excluded from the study. After the two week lead in low-flavonoid diet, subjects came to the lab after a 12 hr overnight fast for their first meal challenge.

MEAL CHALLENGE:

Upon arrival at each meal challenge, each subject was weighed and then completed an infection questionnaire (see Appendix E). Subjects were weighed in kg to insure weight maintenance. A first void urine sample was collected in 100 mL polypropylene containers prior to the meal challenge. A baseline blood draw of ~20 mL was taken from the arm by means of venipuncture with minimum stasis by a trained phlebotomist. Subjects were then provided with a modified McDonald's Big Breakfast® (see Appendix I) as well as either 45g of raisins or 34g of jelly candy. Subjects were instructed to consume all of the meal and treatment. Water was allowed ad libitum for the duration of the test. Postprandial blood draws were completed at 2, 3, and 4 hours after the subject finished the meal and treatment. Subjects remained resting either in a seated or supine position for the entire 4-5 hours on test days. Additionally, just prior to venipuncture, subjects were required to rest quietly ~10 minutes prior to each blood draw.

DISPENSING OF TREATMENT AND STORAGE: RAISIN AND JELLY CANDY

A total of 38.1 kg (83.8 lbs) of Thompson Seedless raisins were provided by the California Raisin Board (CRB). Assurance was given that the treatment raisins were harvested from the same region by the CRB. Raisins were mixed and then divided into 45g portions (~134.50 kcal), weighed on food scales (Denver Instruments Company, Arvada, CO). Raisins were stored in labeled baggies in opaque plastic bins at 2.5°C until time of treatment. Raisins were stored at -12.8°C in the lab during controlled feedings periods.

The jelly candy treatments used as placebo were Starburst® Original Jelly Beans. A total of 24 beans were weighed on 3 separate trials and were found to be isocalorically equivalent to the raisin treatment. Approximately 34.15g of jelly beans was equivalent to 130.10 kcal, and 45g of raisins ~134.5 kcal.

TREATMENT:

Prior to the morning of the first meal challenge, each subject was randomly assigned to start a treatment, either raisins or jelly candy. Treatment was designed to be consumed twice daily at the breakfast and dinner meal for fourteen consecutive days. Subjects were provided with days 1-7 of their treatment on the day of their first meal challenge, days 8-14 were given on a meal by meal basis during the controlled feeding period. Subjects were given instructions on consumption as well as storage of their respective treatment (see Appendix H) with explicit instructions to consume all treatment.

CONTROLLED FEEDINGS:

Subjects arrived at 7AM on days 22-28 and 50-56 of their treatments. All subjects were weighed in kg each morning prior to the breakfast meal. Weights were tracked to insure appropriate kcal diet for weight maintenance. Subjects consumed the breakfast and dinner meals in the lab and were given a take-away lunch packed in a portable cooler. All food items pre and post meals (waste) were weighed on food scales (Denver Instrument Company XP-1500, Arvada, CO) in order to track energy intake. On the final day of the controlled feeding, days 28 and 56, subjects returned to the lab for the second meal challenge test. Subjects repeated the same meal challenge and treatment test following the same methodology.

CONTROLLED FEEDING MENUS:

Diet Menus: A 3 day rotating diet was designed for controlled feeding periods. Menus were analyzed for nutritional composition via Nutrition Data System for Research (NDS-R version 5.0_35), Nutrition Coordinating Center, University of Minnesota, Minneapolis, MS. Meals were modeled after a standard western diet. All menus complied with American Heart Association (AHA) macronutrient recommendations of 50% carbohydrate, 30% Fat, and 15% protein (see Appendix I for sample menu) in order to reduce any confounding variables due to macronutrient distribution. In addition, diets were analyzed for antioxidant vitamin C, E, and A. Menus were adjusted to accommodate multiple calorie levels; 1700, 2000, 2500, 3000, and 3500 kcal/day. Subject caloric needs for weight maintenance were estimated using a modified Harris-Benedict Equation for sedentary Overweight adults (ADA). Each subject was assigned a diet level that best matched their caloric needs. Adjustments for weight loss were made using 100 kcal carbohydrate snacks. No adjustments for weight gain were necessary during the controlled feeding periods.

FOOD PROCUREMENT:

Total grocery needs were purchased immediately prior to the start of the controlled feeding week. The majority of the food was purchased at a local grocery chain, specialty items were purchased at a large national super-store. All food items and meals were stored and prepared in the lab. Periodic inventories were conducted in order to avoid any unexpected deviations from the menu and to eliminate expired items.

WASHOUT AND CROSSOVER:

A two week washout period followed the two weeks of treatment. Subjects were reminded to continue on the low-flavonoid during this time. Following the washout period subjects returned to the lab to repeat the investigation consuming the alternate treatment.

24 HOUR RECALL:

Two unannounced 24 hr recall were conducted via phone interviews in order to assess diet compliance. One recall was conducted during the two week lead-in period, the second during the two week washout period between treatments. Recalls were randomly conducted on

one week day and one weekend day when possible. Records were analyzed via Nutrition Data System for Research (NDS-R version 5.0_35), Nutrition Coordinating Center, University of Minnesota, Minneapolis, MS.

URINE COLLECTION AND ANALYSIS:

Subjects provided first-void urine samples after an overnight 12 hr fast on the mornings of the CRP screening and all four meal challenges. Urine samples were collected in 100 mL polypropylene containers and were stored at 5°C prior to being aliquoted into cryovials for storage at -80°C until analysis of creatinine and urinary isoprostanes (8-epi PGF_{2α}).

On the days where analyses were performed, all appropriate samples were thawed to room temperature and vortexed to mix thoroughly. Urinary creatinine concentrations were analyzed in duplicate via spectrophotometry using a commercially available colorimetric kit (Creatinine Procedure #0400, Stanbio Laboratory, Boerne, TX). Creatinine quantification was necessary in order to normalize isoprostanes levels. In the situation of duplicates having a CV% greater than 10%, or necessary adjustments for divergent concentrations, samples were reanalyzed. Urinary Isoprostanes were analyzed via ELISA using a commercially available kit (Neogen Corporation, Lexington, KY). Duplicates with a CV% greater than 10% were reanalyzed. Urinary isoprostanes were then determined and reported in ng/mg creatinine.

BLOOD COLLECTION AND ANALYSIS:

On the mornings of the meal challenges, after a 12 hr overnight fast, subjects reported to the lab and a baseline blood draw of ~20 mL was taken from the arm by means of venipuncture with minimum stasis by a trained phlebotomist. After blood samples were collected from each subject in two 10 mL serum separator tubes (heparinized), samples were centrifuged (International Equipment Company Centra MP4R, Needham Heights, MA) at 2500 rpm for 15 minutes at 4°C. The serum was immediately drawn and aliquoted into cryovials which were frozen at -80°C until analysis for all measures. Serum ORAC_{total}, ORAC_{pca} and urinary isoprostanes samples were all flushed with nitrogen gas, flash frozen in liquid nitrogen and stored at -80°C.

Serum samples designated for ORAC_{total} and protein free plasma (ORAC_{pca}) assay were collected after centrifugation and aliquoted (1 mL) into a single cryovial. A 100 µL aliquot of serum was stored at -80°C in a microcentrifuge tube for analysis of ORAC_{total}.

The reagent for the protein free procedure, 0.5 M perchloric acid (PCA), was prepared prior to analysis. PCA was prepared by weighing 7.1757 g (70% PCA) into a 100 mL volumetric flask, brought up to volume with distilled water and stored at 4°C until time of analysis. Samples for ORAC_{pca} (150 µL sample: 150 µL PCA) were aliquoted in duplicate into microcentrifuge tubes. Samples were vortexed in order to mix thoroughly, centrifuged at 3000 rpm for 10 minutes. A 150 µL aliquot of the supernatant was stored in a separate microcentrifuge tube at -80°C.

ORAC_{total} and ORAC_{pca} were analyzed shipped on dry ice to the United States Department of Agriculture (USDA) Jean Mayer Human Nutrition Research Center on Aging Antioxidants Research Lab, located at Tufts University, Boston, MA. All ORAC analyses were carried out by Oliver Chen. The ORAC assay was conducted according to modified methods of (120) and (121). Briefly, the ORAC assay provides an integrated and quantitative determination of "total antioxidant capacity" by employing the area under the curve (AUC) of the magnitude and time to the oxidation of fluorescein due to peroxyl radicals generated by the addition of 2,2'-azobis (2-amidinopropane) dihydrochloride. Briefly, fluorescein (FL) stock solution (10 µmol/L) was prepared with phosphate buffer (0.75 M, pH ~7.0) (122) and aliquots were stored in brown cryogenic tubes at -80°C. FL working solutions were always shielded from light and diluted immediately before use with buffer to a final concentration of 200 nmol/L. Aliquots of a Trolox standard stock solution (100 µmol/L) in buffer were stored at -80°C and diluted to 5-50 µmol/L for calibrating standard curves. Plasma samples for ORAC assay (ORAC_{total}) were diluted directly with buffer. ORAC assays of protein-precipitated plasma (ORAC_{pca}) were first treated with 0.5 M perchloric acid (PCA) (1:1 v/v) prior to dilution with buffer. Duplicate blanks, standards, and samples (50 µL each) were placed in a polypropylene, black 96 well plate using reciprocal dosing order across the rows. One hundred µL of FL working solution was added to each well with a multi-pipette. AAPH in buffer (37°C) (0.32 mol/L) was delivered by an automated syringe controlled by the plate reader. The prepared plate was placed in preheated (37°C) microplate reader and incubated for 10 min with 10 sec orbital shaking every 2 min prior to the addition of 25 µL AAPH. Fluorescence intensity was detected at 485/520 (ex/em) every 2

min for 60 min. Values for $ORAC_{total}$ and $ORAC_{pca}$ were calculated according the method described by (121). Net area under the FL decay curve of each standard and sample were calculated by subtracting AUC_{blank} from AUC_{sample} . The Trolox Equivalent (Trolox Eq.) of each sample was calculated with the Trolox standard curve from the net $AUC_{standard}$.

On the days that additional analyses were performed in the lab at Virginia Tech, all appropriate serum samples were allowed to thaw to room temperature. Each sample was vortexed in order to mix thoroughly.

Serum samples analyzed for C-Reactive Protein (CRP) concentrations were quantified in duplicate via Enzyme-Linked Immunosorbent Assay (ELISA) using a commercially available kit (United Biotech Incorporated, Mountain View, CA). Duplicates with a CV% greater than 15% were reanalyzed.

Serum samples for soluble Intercellular Adhesion Molecule-1 (sICAM-1) concentrations were quantified in duplicate via Enzyme-Linked Immunosorbent Assay (ELISA) using a commercially available kit (R&D Systems, Minneapolis, MN). Duplicates with a CV% greater than 10% were reanalyzed.

Serum samples for soluble Vascular Cell Adhesion Molecule-1 (sVCAM-1) concentrations were quantified in duplicate via Enzyme-Linked Immunosorbent Assay (ELISA) using a commercially available kit (R&D Systems, Minneapolis, MN). Duplicates with a CV% greater than 10% were reanalyzed.

Serum samples for cytokine Interleukin-6 (IL-6) concentrations were quantified in duplicate via Enzyme-Linked Immunosorbent Assay (ELISA) using a commercially available kit (R&D Systems, Minneapolis, MN). Duplicates with a CV% greater than 10% were reanalyzed.

(Change) Serum samples for insulin concentrations were quantified in duplicate via Enzyme-Linked Immunosorbent Assay (ELISA) using a commercially available kit (Diagnostic Systems Laboratory, Webster, TX). Duplicates with a CV% greater than 10% were reanalyzed.

Serum samples for triacylglycerol (TAG) concentrations were quantified in duplicate via spectrophotometry using a commercially available enzymatic-colorimetric kit (Wako Chemicals USA, Richmond, VA). The adapted microplate procedure was employed for multiple sample analysis (Wako Chemicals USA, Richmond, VA). Duplicates with a CV% greater than 10% were reanalyzed.

Serum samples for Non-esterified Fatty Acids (NEFA), also known as Free Fatty Acids (FFA) concentrations were quantified in duplicate via spectrophotometry using a commercially available enzymatic-colorimetric kit (Wako Chemicals USA, Richmond, VA). The adapted microplate procedure was employed for multiple sample analysis (Wako Chemicals USA, Richmond, VA). Duplicates with a CV% greater than 10% were reanalyzed.

Serum samples for glucose concentrations were quantified in duplicate via spectrophotometry using a commercially available enzymatic-colorimetric kit (Stanbio, Duplicates with a CV% greater than 10% were reanalyzed.

RAISIN EXTRACTION:

A modified extraction of pigments was used as outlined by Hong and Wrolstad (123) and Karadeniz et al. (27). Samples were chosen for analysis represented significant time points during the project (Table 10). Samples of frozen (-80°C) material were flash frozen in liquid nitrogen and blended for 30 seconds (120v at 25%) using a Waring 250mL stainless steel mini-sample container (Waring, Torrington, CT). Samples were further blended with 50mL acetone for 120 seconds. The acetone portion was decanted and filtered using a fluted filter (C802 grade) and a funnel/ 500 mL separatory funnel. Sample residue was blended with aqueous acetone (70:30) and decanted into the separatory funnel. Both phases were decanted into 100mL glass storage tubes; the darker phase was frozen at -12°F until further analysis. The clear phase was evaporated (Rotavapor R-3000, Buschi, Switzerland), and stored at -12°F until further analysis.

TOTAL PHENOLIC EXTRACTION:

Samples were analyzed by the Folin-Ciocalteu method by Spanos and Wroltsrad (111). All samples were brought to room temperature and then centrifuged for 10 minutes at 1500 RPM (IEC, International Centrifuge, Model PR-2) and again for 10 minutes at 3000 RPM. A standard curve was prepared using Gallic acid stock (1.0g/L). 100 µL aliquots of samples and standards were mixed with 900 µL DI water and 5.0 mL of Folin-Ciocalteu reagent was added to each tube. After vortexing, 4 mL of saturated sodium carbonate was added to each tube then vortexed. All samples and standards incubated for 2 hour at room temperature and then were read at 765 nm (Spec?) All samples and standards were prepared in duplicate.

Appendix B

Individual Values for Lab Measurements

Note: All means and standard deviations in Appendix B based on non-transformed raw data.

Table B1. Raw Data for Descriptives								
Subject #	Gender	Age	Height (cm)	Weight (kg)	BMI	Waist Cir (cm)	Hip (cm)	WHR
1	M	39	176.5	107.7	35	127.0	114.3	1.11
2	F	33	160.0	108.5	42	114.3	134.6	0.85
3	F	20	158.5	100.5	40	114.3	130.8	0.87
4	F	36	157.0	96.0	38	111.8	132.1	0.85
5	F	18	154.0	72.0	30	91.4	104.1	0.88
6	M	22	184.2	106.0	31	95.3	121.9	0.78
7	M	28	182.5	134.0	40	113.7	123.8	0.92
8	M	22	187.3	95.8	27	102.9	113.0	0.91
9	M	40	171.0	105.4	36	116.8	114.3	1.02
10	M	28	175.0	78.8	26	94.0	101.6	0.93
11	F	21	170.0	122.5	42	129.5	137.2	0.94
13	F	18	161.3	70.4	27	94.0	108.0	0.87
15	M	26	182.9	94.4	28	102.9	111.1	0.93
17	M	36	169.0	81.1	28	101.6	102.9	0.99
18	F	21	170.8	78.7	27	95.3	113.0	0.84
19	F	21	163.8	72.5	27	95.3	105.4	0.90
20	F	21	167.0	127.4	45	125.7	146.1	0.86
Mean		26	170.0	97.2	33	107.4	118.5	0.91
STD		8	10.2	19.7	7	12.7	13.5	0.08

Table B2. Raw data for Subject Weights (kg) (#1-9)									
Subject #	1	2	3	4	5	6	7	8	9
Screening	107.7	108.5	100.5	96.0	72.0	106.0	134.0	95.8	105.4
Controlled Feeding (CF) Week 1									
CF1	109.0	109.0	102.0	97.0	73.4	107.0	135.0	96.6	
CF2	108.4	109.6	101.8	95.8	73.6	107.0	134.7	95.9	104.1
CF3	108.2	110.6	102.0	96.0		107.6		95.6	103.3
CF4		110.0	101.8		73.7			95.6	102.5
CF5	108.5	110.9	100.7	97.3	73.5	106.0	133.8	95.0	102.1
CF6	108.5	110.2		97.1	72.9	106.7	134.0	94.1	103.4
CF7	107.8		100.9	96.5	72.6	106.6	134.0	93.9	103.1
Mean	108.3	109.8	101.4	96.5	73.1	106.7	134.3	95.3	103.4
STD	0.4	0.9	0.7	0.6	0.6	0.6	0.5	0.9	1.1
Controlled Feeding (CF) Week 2									
CF8	108.1		102.6	96.3	72.4	107.7	130.8	93.2	104.4
CF9	108.7		102.0	95.8	72.2	108.2	132.7	94.3	103.9
CF10	108.8	108.8	102.0	95.9	73.2	109.0	133.4	94.3	103.9
CF11	109.4	109.9	101.8	96.2	72.7	109.7	134.2	94.3	102.5
CF12	108.8	109.8	101.8	95.8	73.0	108.2	135.1	93.1	102.5
CF13	108.5	109.8	101.1	95.8	72.6	108.1	132.0	93.3	103.4
CF14	108.9	110.8	103.1	95.8	72.3		131.5	93.3	103.1
Mean	108.7	109.8	102.1	95.9	72.6	108.5	132.8	93.7	103.4
STD	0.4	0.7	0.6	0.2	0.4	0.7	1.5	0.6	0.7
Meal Challenge (MC) Days									
MC1	108.0	109.2	100.7	97.4	72.6	107.9	134.7	95.6	104.6
MC2	107.6	109.7	101.2	96.5	72.0	106.8	135.9	94.3	102.7
MC3	107.9	109.7	102.6	96.9	72.8	107.3	130.3	95.3	104.3
MC4	107.7	110.1	100.9	96.1	71.3	107.7	134.1	93.3	102.4
Mean	107.8	109.7	101.4	96.7	72.2	107.4	133.8	94.6	103.5
STD	0.18	0.37	0.86	0.56	0.68	0.49	2.42	1.06	1.11

Table B3. Raw Data for Subject Weights (kg) (#10-20)								
Subject #	10	11	13	15	17	18	19	20
Screening	78.8	122.5	70.4	94.4	81.1	78.7	72.5	127.4
Controlled Feeding (CF) Week 1								
CF1	79.7	123.6	71.1	97.1	80.9	76.9	72.0	129.4
CF2	79.2	123.2	70.3	96.8	80.7	77.6	72.3	129.5
CF3	79.0	122.0	70.4	95.7	80.2	77.0	72.3	128.1
CF4	78.8	122.1	71.2	97.7	80.1	76.9	71.3	128.6
CF5	79.1	121.6	69.5	96.9	80.2	76.5	71.5	127.2
CF6	79.2	121.8	70.3	95.8	80.4	77.3	71.7	127.5
CF7	79.2	121.0	69.9	96.0	79.9	77.6	71.9	128.5
Mean	79.1	122.2	70.4	96.3	80.4	77.3	71.9	128.3
STD	0.3	0.8	0.6	1.0	0.4	0.7	0.4	0.9
Controlled Feeding (CF) Week 2								
CF8	79.8	122.9	69.3	97.2	79.6	77.3	71.7	130.0
CF9	78.9	121.9	69.3	96.9	79.4	77.7	71.4	129.6
CF10	78.7	121.2		95.7	79.3	76.8	71.6	129.5
CF11	78.5	120.5		96.4	79.3	76.3	70.8	128.5
CF12	77.7	120.7	69.0	95.7	78.9	76.0	71.1	129.5
CF13	78.5	120.0	68.9	95.3	78.9	76.0	71.4	128.8
CF14	78.2	120.4	69.0	94.8	78.8	75.9	71.2	128.6
Mean	78.6	121.1	69.1	96.0	79.2	76.6	71.3	129.2
STD	0.6	1.0	0.2	0.9	0.3	0.7	0.3	0.6
Meal Challenge (MC) Days								
MC1	80.3	123.8	70.2	95.6	80.1	76.3	72.8	128.0
MC2	79.7	121.3	69.9	96.1	79.8	78.0	71.4	126.9
MC3	79.7	121.7	69.0	98.0	79.5	78.9	72.7	128.3
MC4	79.3	120.5	69.6	95.5	78.4	77.2	71.4	127.9
Mean	79.8	121.8	69.7	96.3	79.5	77.6	72.1	127.8
STD	0.41	1.39	0.51	1.17	0.74	1.11	0.77	0.61

Table B4. Raw Data for Four Day Food Records (Average)								
Subject	Total kcal	%FAT	%CHO	%PRO	Vit C (mg)	Vit E (IU)	Vit A (RAE)	#Days
1	2905	38.97	50.97	12.3	45.15	16	546	4
2	2816	44.73	42.64	13.3	47.28	12	1410	4
3	3473	36.87	51.57	12.65	95.64	15	1111	4
4	2068	43.4	37.97	19.04	12.86	11	405	4
5	2617	40.85	43.12	16.56	84.94	12	971	4
6	2266	35.85	39.95	19.87	199.22	8	337	4
7	3630	46.43	36.83	19.83	279.53	93	1087	4
8	2297	38.95	45.27	16.42	44.53	11	786	3
9	2156	53.91	24.34	20.95	17.31	8	597	4
10	2910	56.69	25.3	16.49	27.64	9	1167	3
11	1511	32.76	55.06	14.79	82.75	10	715	4
13	2475	36.29	50.05	15.66	144.39	18	1004	4
15	3022	31.05	50.35	15.26	377.96	15	622	4
17	2324	40.16	46.12	14.49	213.85	10	331	4
18	1851	35.26	53.91	12.18	22.97	10	374	4
19	1551	35.62	47.74	12.62	16.84	7	412	4
20	2308	45.43	43.75	11.97	56.89	17	466	4
Mean	2481	41	44	16	104	17	726	4
STD	579	7	9	3	102	19	329	0

Table B5. Raw Data for Individual Subject 24 hr Recall*								
Subject	24hr kcal	%FAT	%CHO	%PRO	Vit C (mg)	Vit E (IU)	Vit A (RAE)	#Days
1	2015	34.01	46.49	19.92	32.88	5	628	3
2	1871	36.55	47.22	17.22	32.92	7	442	5
3	3299	46.89	42.1	11.37	22.13	10	1168	3
4	2752	48	32.35	19.85	49.48	12	667	3
5	1700	49.95	29.64	20.26	37.39	8	547	2
6	1884	37.36	49.99	13.06	71.95	7	87	2
7	1820	30.15	27.34	40.74	61.84	7	816	3
8	2380	41.49	48.34	11.05	35.34	11	506	2
9	1935	49.12	17.91	31.61	19.17	11	495	2
10	2922	45.07	37.88	17.61	16.42	13	598	2
11	1697	37.62	40.81	22.45	33.81	10	317	2
13	2184	45.09	41.2	16.4	86.35	19	1053	2
15	2964	36.77	40.44	20.84	44.37	12	607	2
17	2100	39.91	48.32	12.18	12.81	8	310	2
18	828	28.68	57.18	15.2	31.73	8	212	2
19	1034	23.65	61.18	19.65	14.7	13	580	2
20	1610	36.91	55.63	9.41	78.13	14	328	2
Mean	2059	39	43	19	40	10	551	2
STD	636	7	11	8	22	3	270	1

* Averages include both 24 hr Recall (2 d) and records obtained from subject absences

Table B6. Raw Data for Urinary 8-iso-Prostagandin F2α pg/mg creatinine					
Subject	Screening	Pre Raisin Treatment Baseline	Post Raisin Treatment Baseline	Pre Placebo Treatment Baseline	Post Placebo Treatment baseline
1	3225.118	3729.484	3403.636	4643.390	2143.037
2	3924.817	4490.802	4504.308	6666.632	3126.702
3	6947.263	5251.254	4434.378	7966.763	5665.324
4	4923.022	7832.403	4676.331	3611.867	2305.333
5	5035.170	5222.338	4771.645	4938.135	2860.231
6	5845.807	8884.455	4931.827	4842.620	5859.076
7	5645.196	3115.501	3618.508	160611.233*	215415.116*
8	3310.093	5246.272	4002.194	4713.387	4012.436
9	3552.000	2944.979	2760.000	3194.601	2363.236
10	2729.522	2646.101	6154.752	3037.067	2958.116
11	2799.367	3558.521	2093.622	4080.247	1578.730
13	3169.901	4451.213	3997.382	2987.574	3250.958
15	4785.787	4645.529	2738.773	4567.785	2898.509
17	3721.932	3853.950	3668.329	3969.969	3508.026
18	3383.555	2637.146	2174.667	2961.333	2819.800
19	5195.504	3415.189	2378.269	4140.814	3464.417
20	4428.193	4767.152	2409.905	4773.501	3009.099
Mean	4271.897	4511.311	3689.325	4443.480	3238.939
STD	1203.683	1702.583	1151.910	1341.406	1144.529
				*excluded as outlier	

Table B7. Raw Data for Urinary Creatinine mg/dL					
Subject	Screening	Pre Raisin Treatment Baseline	Post Raisin Treatment Baseline	Pre Placebo Treatment Baseline	Post Placebo Treatment baseline
1	193.512	131.111	106.111	172.222	91.667
2	267.299	306.362	236.607	89.049	146.571
3	208.140	143.204	151.092	163.228	337.209
4	60.268	116.505	53.571	79.490	95.267
5	383.721	46.205	202.907	170.510	315.534
6	323.480	331.081	347.973	284.598	331.926
7	105.435	216.518	299.107	302.455	165.179
8	104.891	88.043	128.804	147.283	304.129
9	46.875	88.587	219.022	102.174	202.174
10	274.554	214.286	318.412	310.268	195.313
11	197.545	235.491	160.870	158.152	226.004
13	109.783	55.580	98.214	367.188	177.455
15	252.790	303.013	242.746	270.647	301.339
17	159.783	210.379	166.304	129.348	218.192
18	216.518	247.768	53.571	75.000	108.696
19	140.217	80.435	109.239	61.413	286.830
20	235.491	206.473	260.045	277.902	268.973
Mean	192.959	177.708	185.564	185.937	221.909
STD	92.524	91.352	90.057	96.570	83.471

Table B8. Raw Data for Serum Total Oxygen Radical Antioxidant Capacity (ORAC_{total})					
uM Trolox Equivalent (TE)					
Subject	Screening	Pre Raisin Treatment Baseline	Post Raisin Treatment Baseline	Pre Placebo Treatment Baseline	Post Placebo Treatment baseline
1	9735	8809	9467	7289	9011
2	9136	9363	8683	8152	8204
3	6790	4760	5369	6131	6837
4	6504	7190	6567	6841	6354
5	8982	7196	8185	8495	9921
6	9426	8522	9140	5863	5667
7	9243	9225	7643	8161	7954
8	7046	6165	6994	6491	7260
9	6881	8456	7592	9561	8511
10	7949	8095	7771	5812	6555
11	8451	7943	7838	7896	8379
13	8824	7571	8521	8920	8310
15	7724	8784	8045	7716	8906
17	7535	7749	7962	6436	6781
18	7535	6436	6781	7749	7962
19	10922	10617	11837	13132	12455
20	9982	11204	11329	9477	8859
Mean	8392.017	8122.699	8219.098	7889.579	8113.268
STD	1273.564	1578.027	1601.974	1793.365	1582.510

Table B9. Raw Data for Serum Oxygen Radical Antioxidant Capacity (ORAC_{pca}) perchloric acid (PCA) uM Trolox Equivalent (TE)					
Subject	Screening	Pre Raisin Treatment Baseline	Post Raisin Treatment Baseline	Pre Placebo Treatment Baseline	Post Placebo Treatment baseline
1	1258	1073	1169	1043	996
2	1057	980	983	929	1044
3	1115	784	847	843	925
4	948	1004	1051	1013	1002
5	974	881	900	986	1053
6	943	899	1089	717	760
7	1397	1185	1182	1119	1166
8	866	839	835	877	1055
9	928	1063	1048	880	919
10	962	1001	1032	798	875
11	1047	1295	1106	1078	1118
13	1089	917	1096	1033	1011
15	1102	1067	1080	991	1111
17	808	897	925	701	794
18	763	652	689	893	774
19	825	842	942	849	837
20	1117	1189	1198	1153	1214
Mean	1011.768	974.575	1010.094	935.565	979.637
STD	163.440	162.221	138.403	132.305	138.145

Table B10. Raw Data for Serum C-Reactive Protein (CRP) mg/L									
Subject	Screening	Pre Raisin Treatment zero hour	2 hours	3 hours	4 hours	Post Raisin Treatment zero hour	2 hours	3 hours	4 hours
1	3.799	7.396	6.629	6.106	6.149	9.849	8.693	9.652	9.851
2		9.147	9.368	9.120	9.106	14.321	14.639	16.438	13.300
3	11.456	10.756	12.939	13.814	11.865	11.814	12.173	11.827	11.239
4	1.367	1.367	1.514	1.593	1.668	1.348	1.538	1.389	1.427
5	5.348	5.285	9.113	8.281	6.277	5.079	4.686	6.210	5.894
6	12.588	21.139	17.849	19.908	17.060	12.631	14.347	13.986	15.699
7	0.410	0.350	0.350	0.715	0.847	0.793	1.606	1.215	1.498
8	0.571	0.350	0.456	0.350	0.350	0.438	0.413	0.805	0.480
9	3.676	1.893	2.174	1.761	2.267	1.474	1.356	0.954	1.707
10	0.252	0.350	1.421	0.666	0.441	1.281	0.828	0.350	1.348
11	16.482	11.489	8.905	8.856	10.656	10.015	9.588	9.761	9.368
13	0.824	0.350	0.557	0.432		25.790*	21.454*	19.822*	19.570*
15	0.350	0.457	0.644	0.378	0.646	0.350	0.350	0.437	0.546
17	0.940	1.258	1.532	1.230	1.024	1.069	1.439	0.916	1.100
18	3.478	0.824	1.213	1.095		0.350	0.350	0.350	
19	12.352	17.240	15.717	14.935	17.365	3.318	3.404	3.737	4.090
20	14.669	17.833	14.700	10.975	16.259	9.106	13.484	8.645	9.454
Mean	5.535	6.323	6.181	5.895	6.799	5.202	5.556	5.417	5.800
STD	5.840	7.093	6.162	6.210	6.483	5.138	5.581	5.527	5.220
Subject		Pre Placebo Treatment zero hour	2 hours	3 hours	4 hours	Post Placebo Treatment zero hour	2 hours	3 hours	4 hours
1		10.933	14.268	14.372	13.405	12.432	11.467	14.284	10.811
2		12.215	14.368	12.599	13.701	12.522	12.128	13.006	10.783
3		13.588	12.880	16.784	15.847	15.938	12.469	12.756	13.901
4		0.833	1.503	1.331	1.128	2.221	2.238	2.600	2.950
5		2.192	3.822	3.358	2.336	2.193	2.243	3.623	3.854
6		13.132	12.484	13.586	11.568	15.366	14.697	15.301	16.593
7		0.504	0.841	0.714	0.350	2.120	1.510	1.040	1.010
8		0.350	0.350	0.899	0.794	0.489	0.350	0.640	0.350
9		1.952	1.588	2.583	1.767	5.596	4.144	4.137	3.960
10		0.586	0.524	0.587	0.350	0.466	1.048	0.400	1.340
11		12.041	11.783	12.723	16.154	13.307	14.747	11.752	10.013
13		0.350	0.350	0.350	0.350	0.369	0.424	0.350	0.350
15		0.557	0.350	0.350	0.350	0.780	0.772	0.486	0.606
17		0.555	0.970	0.769	1.031	0.610	0.660	0.678	0.664
18		0.880	1.059	0.793	1.282	0.350	0.350	0.350	0.350
19		20.906	20.485	18.635	16.117	9.977	10.900	9.451	9.042
20		9.549	10.504	6.194	5.598	11.789	10.215	8.584	10.021
Mean		5.948	6.361	6.272	6.008	6.266	5.904	5.849	5.682
STD		6.657	6.781	6.769	6.636	6.108	5.757	5.750	5.469
*excluded due to infection									

Table B11. Raw Data for Serum Interleukin-6 (IL-6) pg/mL

Subject	Screening	Pre Raisin Treatment zero hour	2 hours	3 hours	4 hours	Post Raisin Treatment zero hour	2 hours	3 hours	4 hours
1	1.335	1.090	1.576	1.185	1.178	1.951	1.106	1.572	0.886
2	1.077	1.415	0.632	1.171	1.171	1.802	1.539	1.492	1.550
3	2.010	1.320	0.933	0.901	1.386	1.061	0.907	0.720	1.059
4	2.892	1.356	0.822	1.011	1.169	0.667	0.545	0.855	0.516
5	1.287	1.965	1.783	1.968	1.422	1.050	1.157	1.254	1.299
6	4.072	5.730	4.635	3.732	3.164	4.278	2.857	2.980	2.871
7	0.626	0.424	0.261	0.218	0.374	0.489	0.557	0.466	0.536
8	0.698	0.795	0.504	0.709	0.629	0.548	0.419	0.653	0.767
9	1.033	1.296	0.641	0.714	0.754	0.771	0.609	0.387	0.722
10	0.859	0.387	0.260	0.334	0.347	0.474	0.368	0.445	0.238
11	1.802	2.241	1.820	1.831	2.477	1.436	1.334	1.769	1.142
13	0.555	0.506	0.659	2.521		5.174*	2.056*	2.503*	3.439*
15	0.986	1.008	0.771	1.112	0.679	1.172	0.608	0.862	0.930
17	0.290	0.608	0.377	0.301	0.334	0.389	0.656	0.254	0.290
18	0.544	0.504	0.152	0.199		0.523	1.291	0.479	
19	1.085	0.890	0.567	0.438	0.490	0.556	0.379	0.261	0.511
20	3.678	3.553	3.579	3.340	2.548	3.110	3.810	3.416	5.045
Mean	1.461	1.476	1.175	1.276	1.208	1.267	1.134	1.117	1.224
STD	1.107	1.356	1.226	1.071	0.880	1.081	0.950	0.943	1.238
Subject		Pre Placebo Treatment zero hour	2 hours	3 hours	4 hours	Post Placebo Treatment zero hour	2 hours	3 hours	4 hours
1		1.995	1.634	1.579	1.758	0.864	1.561	2.021	1.933
2		1.090	1.273	1.236	1.051	1.579	1.645	1.743	2.170
3		1.609	1.115	1.149	1.520	1.161	0.696	0.776	0.867
4		1.152	1.189	1.165	1.577	1.332	1.124	1.006	1.577
5		1.229	0.785	1.418	1.519	1.218	0.821	1.060	1.407
6		5.782	3.941	3.663	3.793	5.351	3.775	4.313	3.565
7		0.500	0.337	0.412	0.377	1.270	0.655	0.760	1.584
8		0.742	0.552	1.089	0.662	0.395	0.346	0.472	1.037
9		0.815	1.526	0.573	0.789	1.498	0.993	0.920	1.126
10		0.518	0.431	1.627	0.402	0.896	0.569	0.392	0.430
11		1.918	2.604	2.128	2.777	1.483	1.316	1.813	1.871
13		0.564	0.819	1.189	7.011	0.793	0.649	0.938	0.714
15		1.228	0.810	1.116	0.870	0.845	0.613	0.246	0.906
17		0.484	0.163	0.369	0.250	0.536	0.158	0.186	0.330
18		1.369	1.266	0.927	0.957	0.960	0.442	0.416	0.431
19		0.916	0.762	0.622	0.453	0.291	0.206	0.323	0.526
20		4.337	2.568	3.063	3.733	3.336	3.494	3.053	4.300
Mean		1.544	1.281	1.372	1.735	1.400	1.121	1.202	1.457
STD		1.425	0.970	0.881	1.743	1.223	1.040	1.105	1.095

*excluded due to infection

Table B12. Raw Data for Serum Soluble Intercellular Adhesion Molecule 1 (sICAM-1) ng/mL

Subject	Screening	Pre Raisin Treatment zero hour	2 hours	3 hours	4 hours	Post Raisin Treatment zero hour	2 hours	3 hours	4 hours
1	281.929	251.492	225.581	221.646	217.279	220.508	233.015	259.594	263.718
2	336.361	253.305	250.931	253.265	243.864	268.348	279.626	292.191	284.731
3	202.970	186.975	181.396	179.332	182.405	202.596	192.653	236.326	226.291
4	224.616	199.024	184.556	195.648	216.603	215.669	215.482	197.522	194.333
5	185.116	198.270	203.141	197.336	212.868	197.149	186.062	188.694	188.130
6	134.433	122.454	114.587	131.672	138.389	150.002	108.941	109.996	106.633
7	179.218	182.369	187.491	187.659	208.963	176.889	183.189	173.227	181.371
8	220.647	223.393	212.058	213.673	246.166	252.272	240.684	232.462	233.281
9	228.964	253.204	220.646	252.663	232.533	231.048	218.721	202.713	205.821
10	211.383	200.087	205.277	220.168	221.053	206.203	201.947	208.142	213.093
11	295.320	298.952	310.182	304.321	281.440	265.426	250.924	246.547	268.908
13	206.733	217.170	201.237	189.855		228.633	212.753	206.733	216.277
15	149.841	165.962	168.026	156.553	147.279	157.682	140.426	132.194	142.526
17	247.546	232.094	217.290	121.520	208.662	200.190	198.155	197.236	201.663
18	228.626	195.203	210.134	210.866		205.164	239.558	223.146	
19	219.909	198.505	196.445	178.487	181.612	203.969	195.567	201.895	218.018
20	263.127	226.981	232.012	199.336	222.099	154.160	236.868	224.123	233.174
Mean	224.514	212.085	207.117	200.824	210.748	207.995	207.916	207.808	211.123
STD	50.721	40.245	40.028	44.405	36.901	35.417	40.842	43.554	44.904
Subject		Pre Placebo Treatment zero hour	2 hours	3 hours	4 hours	Post Placebo Treatment zero hour	2 hours	3 hours	4 hours
1		242.749	247.143	233.390	240.893	240.017	235.569	248.961	257.270
2		268.796	269.946	257.064	273.165	272.792	290.471	307.887	282.106
3		214.549	205.392	204.831	208.198	205.580	218.281	219.023	208.006
4		186.797	175.878	177.767	196.772	230.747	241.705	211.741	187.192
5		161.487	168.883	175.874	177.391	192.264	198.187	177.721	166.366
6		119.499	118.975	122.123	128.205	138.034	112.123	105.032	110.532
7		176.058	181.538	189.467	194.863	166.706	167.543	165.193	168.883
8		219.879	210.407	224.608	224.147	231.228	209.232	212.549	222.160
9		233.559	218.527	234.263	212.931	245.761	248.881	247.363	239.683
10		196.487	194.418	200.910	206.235	197.874	188.528	190.168	195.667
11		291.157	291.508	258.444	250.225	263.156	246.898	252.465	267.336
13		235.132	215.047	199.994	195.019	184.506	191.808	181.289	189.677
15		158.146	160.502	160.132	156.552	148.224	148.980	148.798	145.000
17		249.723	226.060	228.798	220.221	195.202	202.215	204.981	220.581
18		202.219	206.632	255.631	202.776	192.493	195.593	190.255	199.873
19		199.002	203.913	175.173	167.989	174.478	164.996	180.402	196.436
20		231.175	222.270	219.047	226.467	213.142	209.247	214.841	225.804
Mean		210.966	206.885	206.913	204.826	205.424	204.133	203.451	204.857
STD		43.098	40.829	37.519	35.147	38.137	42.444	46.102	43.878

Table B13. Raw Data for Serum Soluble Vascular Cellular Adhesion Molecule 1 (sVCAM-1) ng/mL

Subject	Screening	Pre Raisin Treatment zero hour	2 hours	3 hours	4 hours	Post Raisin Treatment zero hour	2 hours	3 hours	4 hours
1	990.381	926.872	923.886	932.273	919.290	942.517	926.922	959.258	909.847
2	910.216	685.818	675.324	694.385	661.973	726.594	732.149	719.437	729.791
3	765.161	725.060	734.533	701.146	714.457	758.180	839.058	857.555	826.651
4	813.421	784.603	758.180	789.099	820.690	731.415	703.810	702.466	687.722
5	519.787	463.381	455.902	404.902	467.474	476.665	438.464	440.493	467.474
6	570.328	562.184	582.314	506.631	523.624	557.122	492.081	510.450	509.498
7	536.365	482.208	422.023	390.688	437.521	426.541	448.149	435.309	485.108
8	752.123	823.407	749.602	771.123	757.006	869.592	809.948	791.503	854.511
9	672.286	627.205	588.456	571.594	554.264	590.536	563.488	566.540	588.044
10	698.442	703.472	734.394	734.233	761.422	750.786	751.518	679.909	646.005
11	693.696	597.788	589.964	540.016	637.787	680.168	587.007	593.029	578.198
13	495.013	550.581	554.082	565.976		743.826	641.863	601.575	569.400
15	731.992	715.064	699.634	667.030	683.003	716.687	651.677	622.053	637.921
17	789.135	773.381	813.153	807.738	767.443	821.557	733.545	713.067	743.205
18	702.097	707.391	731.999	751.106		827.936	903.048	873.836	
19	570.871	514.980	543.845	511.406	533.421	518.517	511.370	499.607	524.722
20	616.830	596.207	638.612	666.628	570.892	581.608	617.675	597.823	558.332
Mean	695.773	661.153	658.583	647.410	654.018	689.426	667.751	656.701	644.777
STD	136.816	127.525	129.499	148.601	138.371	143.848	151.210	151.795	135.791
Subject	Screening	Pre Placebo Treatment zero hour	2 hours	3 hours	4 hours	Post Placebo Treatment zero hour	2 hours	3 hours	4 hours
1		854.629	873.549	876.940	900.603	949.211	956.181	975.735	949.894
2		716.077	677.042	681.971	697.558	711.713	730.703	706.072	729.199
3		854.297	861.532	900.500	887.208	838.248	868.456	810.786	802.382
4		762.160	774.763	779.369	768.860	770.377	767.920	779.136	727.256
5		460.108	430.945	459.919	472.028	478.202	461.886	465.987	463.952
6		595.056	568.563	523.624	505.638	545.589	510.447	504.704	553.629
7		519.886	441.555	455.589	448.253	457.680	467.023	457.705	489.151
8		802.301	787.427	770.938	801.155	805.451	752.945	747.830	807.289
9		607.075	581.359	593.245	608.098	747.040	751.235	803.473	749.527
10		620.789	620.254	635.996	661.221	656.001	652.253	701.893	764.020
11		628.686	631.181	629.945	648.066	660.847	574.883	563.182	575.549
13		488.950	497.463	492.058	486.551	522.884	605.858	602.962	584.424
15		656.240	640.548	652.606	672.373	728.448	648.946	634.865	646.216
17		814.269	811.868	817.232	798.215	796.634	737.577	771.268	755.657
18		812.199	794.423	808.979	801.732	794.374	840.820	824.689	850.448
19		493.151	523.832	574.413	539.964	571.276	529.649	556.296	523.123
20		630.136	652.358	694.496	650.063	619.102	612.134	607.345	620.779
Mean		665.648	656.980	667.519	667.505	685.475	674.642	677.290	681.911
STD		132.997	141.296	141.647	144.160	138.026	143.255	144.197	137.763

Table B14. Raw Data for Serum Free Fatty Acids (FFA) mEq/L									
Subject	Screening	Pre Raisin Treatment zero hour	2 hours	3 hours	4 hours	Post Raisin Treatment zero hour	2 hours	3 hours	4 hours
1	0.497	0.377	0.153	0.281	0.480	0.477	0.241	0.296	0.492
2	0.394	0.313	0.085	0.086	0.241	0.490	0.140	0.136	0.301
3	0.455	0.412	0.266	0.283	0.215	0.626	0.115	0.276	0.268
4	0.186	0.281	0.027	0.072	0.201	0.408	0.069	0.104	0.169
5	0.792	0.473	0.139	0.161	0.139	0.430	0.079	0.123	0.181
6	0.560	0.183	0.081	0.165	0.197	0.249	0.085	0.197	
7		0.262	0.172	0.314	0.353	0.251	0.181	0.286	0.351
8	0.260	0.253	0.145	0.340	0.550	0.391	0.232	0.428	0.433
9	0.404	0.287	0.251	0.253	0.278	0.235	0.165	0.264	0.384
10	0.143	0.116	0.199	0.402	0.474	0.174	0.251	0.390	0.537
11	0.310	0.397	0.130	0.182	0.313	0.338	0.112	0.316	0.470
13	0.125	0.731	0.100	0.158		0.579	0.178	0.202	0.279
15	0.130	0.261	0.180	0.224	0.341	0.495	0.157	0.247	0.351
17	0.324	0.148	0.324	0.386	0.381	0.360	0.264	0.326	0.379
18	0.256	0.396	0.115	0.108		0.272	0.086	0.146	
19	0.153	0.327	0.124	0.299	0.330	0.189	0.115	0.351	0.354
20	0.526	0.456	0.148	0.187	0.456	0.530	0.204	0.280	0.417
Mean	0.345	0.334	0.155	0.229	0.330	0.382	0.157	0.257	0.358
STD	0.189	0.145	0.073	0.101	0.121	0.139	0.064	0.094	0.106
Subject		Pre Placebo Treatment zero hour	2 hours	3 hours	4 hours	Post Placebo Treatment zero hour	2 hours	3 hours	4 hours
1		0.699	0.218	0.278	0.409	0.580	0.306	0.268	0.407
2		0.480	0.034	0.079	0.234	0.401	0.067	0.143	0.265
3		0.358	0.032	0.170	0.313	0.393	0.062	0.156	0.281
4		0.316	0.042	0.074	0.132	0.363	0.100	0.077	0.139
5		0.311	0.148	0.201	0.321	0.383	0.079	0.176	0.311
6		0.127	0.083	0.085	0.130	0.176	0.071	0.151	0.232
7		0.374	0.155	0.211	0.267	0.246	0.120	0.242	0.360
8		0.223	0.186	0.267	0.456	0.235	0.118	0.314	0.323
9		0.278	0.109	0.183	0.226	0.226	0.136	0.246	0.206
10		0.150	0.134	0.177	0.215	0.136	0.143	0.172	0.204
11		0.197	0.130	0.229	0.261	0.269	0.093	0.182	0.284
13		0.356	0.157	0.314	0.420	0.538	0.131	0.124	0.182
15		0.470	0.140	0.254	0.348	0.425	0.065	0.123	0.279
17		0.331	0.146	0.216	0.247	0.216	0.175	0.300	0.276
18		0.160	0.076	0.093	0.105	0.191	0.074	0.105	0.174
19		0.296	0.076	0.196	0.236	0.354	0.146	0.339	0.485
20		0.348	0.117	0.175	0.324	0.557	0.131	0.237	0.249
Mean		0.322	0.117	0.188	0.273	0.335	0.119	0.197	0.274
STD		0.141	0.053	0.072	0.101	0.138	0.059	0.078	0.087

Table B15. Raw Data for Serum Triacylglycerol (TAG) mg/dL									
Subject	Screening	Pre Raisin Treatment zero hour	2 hours	3 hours	4 hours	Post Raisin Treatment zero hour	2 hours	3 hours	4 hours
1	434.622	373.022	421.504	430.059	475.119	364.467	385.570	435.763	475.689
2	55.198	68.444	101.526	123.000	124.911	76.430	118.067	128.904	126.622
3	112.933	62.170	143.163	152.859	122.059	138.030	162.556	172.252	160.044
4	64.156	117.978	128.395	155.596	184.532	113.348	138.234	179.324	226.035
5	93.093	48.531	96.565	130.132	122.029	36.956	83.568	92.514	127.124
6	31.747	33.087	52.823	58.433	46.418	44.234	54.610	106.227	88.464
7	267.589	109.220	229.362	293.255	225.539	211.887	351.688	375.170	367.441
8	125.057	207.518	227.177	283.972	378.993	107.035	191.681	211.340	240.897
9	47.540	222.976	279.445	366.066	306.491	129.227	209.818	226.266	241.616
10	129.446	118.811	231.609	335.365	384.706	117.714	188.437	246.550	280.541
11	164.315	163.766	203.868	268.311	267.018	106.300	114.130	157.493	190.016
13	56.312	44.869	40.653	47.278		37.642	57.517	58.721	74.380
15	124.971	97.869	95.460	180.379	216.515	70.767	131.596	145.448	193.629
17	111.594	100.376	209.018	222.598	211.970	59.045	122.813	124.584	125.765
18	20.666	35.427	44.283	28.932		45.858	53.348	43.480	
19	173.612	135.991	190.264	205.683	223.568	166.211	206.916	243.921	242.070
20	125.765	87.976	145.250	159.420	160.011	122.222	179.495	230.864	260.977
Mean	125.801	119.296	167.080	202.432	229.992	114.551	161.767	186.989	213.832
STD	100.104	85.852	98.019	114.387	116.198	80.472	93.477	103.315	104.307
Subject		Pre Placebo Treatment zero hour	2 hours	3 hours	4 hours	Post Placebo Treatment zero hour	2 hours	3 hours	4 hours
1		227.578	245.259	297.733	296.022	263.511	284.044	372.203	375.874
2		77.000	49.052	51.904	51.904	41.637	58.748	41.013	62.170
3		35.799	70.522	85.569	98.880	112.612	125.507	127.238	116.242
4		92.514	38.692	53.739	91.935	143.442	204.788	223.307	218.677
5		83.833	129.553	155.017	55.198	52.003	80.361	94.829	128.974
6		27.863	12.014	39.472	48.760	21.278	37.313	76.042	94.617
7		145.809	197.142	269.773	224.447	167.652	216.517	300.355	298.716
8		145.809	261.353	323.304	363.873	125.390	166.016	202.691	236.682
9		105.653	203.788	212.560	159.381	111.136	185.148	230.651	191.727
10		91.947	163.218	214.204	209.270	138.734	196.112	240.520	247.099
11		162.914	149.061	154.482	176.766	99.675	132.800	132.800	132.198
13		68.960	91.244	108.549	86.795	31.294	37.789	36.608	43.103
15		82.210	108.107	173.152	218.322	80.403	108.709	126.175	189.413
17		116.318	211.293	217.874	236.178	105.099	180.086	209.608	211.970
18		50.881	53.965	70.237	55.726	35.989	44.697	39.472	41.214
19		126.123	187.181	240.220	206.916	111.938	135.374	203.833	214.776
20		113.956	187.171	247.397	275.738	173.591	237.950	244.444	253.668
Mean		103.245	138.742	171.482	168.007	106.787	143.056	170.693	179.831
STD		49.597	76.614	91.018	96.916	62.017	74.667	96.750	92.765

Table B16. Raw Data for Serum Glucose mg/mL									
Subject	Screening	Pre Raisin Treatment zero hour	2 hours	3 hours	4 hours	Post Raisin Treatment zero hour	2 hours	3 hours	4 hours
1	126.875	110.313	84.375	88.438	121.250	93.125	109.375	113.125	124.375
2	95.000	94.219	88.594	90.625	91.719	92.813	99.219	96.719	94.531
3	94.012	99.102	109.880	118.413	100.449	90.719	91.617	94.311	91.617
4	96.856	101.946	87.575	98.653	94.162	98.503	95.509	100.898	101.796
5	91.038	100.000	121.698	123.428	103.145	89.308	108.176	91.981	89.151
6	90.881	80.818	93.396	86.006	88.836	89.780	92.453	94.969	88.994
7	89.937	94.025	82.075	101.101	95.440	85.220	92.138	103.459	102.516
8	91.824	103.145	87.421	105.189	119.340	88.836	97.956	94.182	102.987
9	90.586	102.315	130.864	131.944	129.012	93.827	113.735	110.957	120.525
10	91.821	85.957	93.981	109.105	120.525	83.951	112.191	100.617	108.796
11	91.512	99.228	116.667	102.160	102.778	85.494	82.253	85.802	91.667
13	93.364	87.963	99.691	91.358		84.568	93.519	90.586	89.352
15	89.969	89.352	91.975	102.315	104.167	87.346	91.667	112.191	105.247
17	92.879	92.424	93.182	108.788	112.727	89.394	95.758	104.848	114.545
18	107.879	92.576	73.788	72.576		90.152	58.788	76.212	
19	126.515	80.000	91.818	95.152	85.758	84.697	78.939	89.091	85.303
20	93.939	97.273	114.091	77.121	85.606	94.394	96.364	105.000	104.242
Mean	97.346	94.744	97.710	100.139	103.661	89.537	94.686	97.938	100.978
STD	11.805	8.177	15.535	15.611	13.997	4.089	13.289	9.912	11.807
Subject	Screening	Pre Placebo Treatment zero hour	2 hours	3 hours	4 hours	Post Placebo Treatment zero hour	2 hours	3 hours	4 hours
1		108.125	74.375	93.125	86.875	102.813	144.688	103.438	108.125
2		94.063	97.188	89.375	92.188	91.719	75.938	88.906	88.906
3		101.048	81.437	80.240	89.970	86.228	83.533	94.162	86.377
4		100.749	91.467	102.545	92.216	92.216	80.389	93.563	97.455
5		92.610	99.686	90.094	88.836	89.151	77.830	81.447	88.994
6		93.553	95.283	100.472	86.321	80.346	93.868	97.170	89.623
7		97.484	103.459	102.673	91.195	83.805	83.333	96.698	98.742
8		84.906	86.164	86.321	113.994	89.151	88.365	92.138	107.075
9		104.475	92.284	91.358	97.994	98.920	113.272	106.790	99.074
10		89.198	89.352	92.284	91.667	85.648	101.389	93.210	102.623
11		99.537	95.679	98.302	93.673	90.432	96.296	93.364	89.352
13		88.272	77.778	92.901	86.420	78.858	93.056	84.259	89.660
15		83.488	101.543	93.519	107.870	85.957	99.074	85.031	103.241
17		86.970	95.000	83.485	103.939	82.727	81.061	102.273	102.727
18		84.697	75.152	80.606	93.030	86.667	89.242	87.727	87.121
19		81.364	81.667	85.758	104.242	86.970	84.091	84.242	92.273
20		88.182	127.273	116.667	95.606	86.970	92.879	91.212	89.545
Mean		92.866	92.046	92.925	95.061	88.151	92.841	92.684	95.348
STD		7.930	12.851	9.212	8.008	6.035	16.435	7.137	7.393

Table B17. Raw Data for Serum Insulin uIU/mL									
Subject	Screening	Pre Raisin Treatment zero hour	2 hours	3 hours	4 hours	Post Raisin Treatment zero hour	2 hours	3 hours	4 hours
1	15.335	12.000	50.414	22.970	21.112	13.075	52.120	30.524	35.483
2	7.553	6.965	24.924	18.454	10.695	6.000	33.233	29.683	13.606
3	22.546	25.369	88.776	164.860	85.081	15.370	59.859	39.929	40.721
4	6.257	7.182	36.875	21.054	12.566	6.000	24.870	19.806	19.541
5	8.616	16.742	113.270	119.590	59.480	7.031	74.896	25.517	16.746
6	6.000	6.000	17.183	10.911	6.000	6.000	16.885	7.028	6.000
7	7.367	7.269	16.260	13.323	6.320	6.000	26.272	12.728	10.151
8	6.000	6.000	15.001	10.786	8.238	6.000	6.581	7.842	7.671
9	6.000	13.907	69.669	56.252	42.658	6.426	32.598	23.024	25.080
10	6.000	7.659	30.042	23.616	10.291	6.000	29.759	12.809	9.011
11	29.584	34.984	163.080	94.109	80.269	20.114	29.007	19.304	15.833
13	12.965	6.000	55.291	20.568		6.3207	45.347	26.468	16.777
15	7.842	6.000	28.564	18.093	9.141	8.580	19.442	29.242	6.000
17	6.849	6.000	21.563	22.085	23.270	6.000	18.713	27.351	22.428
18	6.000	6.000	19.466	11.081		6.000	6.000	8.875	
19	32.579	6.000	13.570	8.122	6.000	6.000	8.056	9.664	12.225
20	37.069	38.622	205.000	78.329	59.165	28.773	116.180	132.170	45.655
Mean	13.209	12.512	56.997	42.012	29.352	9.393	35.283	27.174	18.933
STD	10.515	10.545	55.940	45.649	28.341	6.461	28.129	28.703	12.227
Subject	Screening	Pre Placebo Treatment zero hour	2 hours	3 hours	4 hours	Post Placebo Treatment zero hour	2 hours	3 hours	4 hours
1		13.500	40.394	40.450	18.166	12.000	56.262	46.094	39.129
2		7.597	26.449	10.250	6.877	7.667	18.591	11.207	6.182
3		13.370	87.819	43.003	24.765	17.629	51.110	53.339	17.705
4		9.794	33.514	22.005	13.981	7.105	31.549	25.700	18.311
5		6.173	42.840	24.580	11.133	6.000	67.325	26.143	17.260
6		6.000	15.941	21.992	6.000	6.000	18.386	14.364	7.109
7		10.191	37.574	19.286	8.024	8.056	18.833	12.065	
8		6.000	6.257	6.000	6.000	6.000	8.720	6.069	6.137
9		6.000	34.140	15.786	11.48	6.850	38.562	37.257	12.548
10		7.035	17.490	15.411	8.986	6.000	31.544	14.362	8.544
11		38.152	84.387	44.160	47.539	25.435	72.470	40.084	22.718
13		7.084	25.309	18.003		6.000	36.403	28.933	13.706
15		6.000	18.778	7.748	6.994	6.000	22.451	7.930	10.967
17		6.000	29.613	22.258	23.391	6.000	14.016	24.969	23.405
18		6.000	12.863	22.933	10.275	6.000	15.924	7.099	8.235
19		6.000	12.498	6.000	21.930	6.000	10.102	8.114	12.937
20		23.929	205.000	205.000	62.401	38.569	123.010	91.473	60.777
Mean		10.519	42.992	32.051	17.996	10.430	37.368	26.777	17.854
STD		8.498	47.497	46.105	15.945	8.962	29.505	22.146	14.225

Table B18. Percent Change C-Reactive Protein (CRP) %

Subject	Pre Raisin Treatment zero hour	2 hours	3 hours	4 hours	Post Raisin Treatment zero hour	2 hours	3 hours	4 hours
1	100.000	89.630	82.558	83.140	100.000	88.263	98.000	100.020
2	100.000	102.416	99.705	99.552	100.000	102.221	114.782	92.871
3	100.000	120.296	128.431	110.311	100.000	103.039	100.110	95.133
4	100.000	110.753	116.533	122.019	100.000	114.095	103.042	105.861
5	100.000	172.431	156.689	118.770	100.000	92.262	122.268	116.046
6	100.000	84.436	94.177	80.704	100.000	113.586	110.728	124.289
7	100.000	100.000	204.286	242.000	100.000	202.522	153.216	188.903
8	100.000	130.286	100.000	100.000	100.000	94.292	183.790	109.589
9	100.000	114.844	93.027	119.757	100.000	91.995	64.722	115.807
10	100.000	406.000	190.286	126.000	100.000	64.637	27.322	105.230
11	100.000	77.509	77.082	92.750	100.000	95.736	97.464	93.540
13	100.000	159.143	123.429					
15	100.000	140.919	82.713	141.357	100.000	100.000	124.857	156.000
17	100.000	121.781	97.774	81.399	100.000	134.612	85.688	102.900
18	100.000	147.209	132.888		100.000	100.000	100.000	
19	100.000	91.166	86.630	100.725	100.000	102.592	112.628	123.267
20	100.000	82.431	61.543	91.174	100.000	148.078	94.937	103.822
Mean	100.000	132.426	113.397	113.977	100.000	109.246	105.847	115.552
STD	0.000	75.745	39.350	39.720	0.000	31.219	34.341	25.834
Subject	Pre Raisin Treatment zero hour	2 hours	3 hours	4 hours	Post Raisin Treatment zero hour	2 hours	3 hours	4 hours
1	100.000	130.504	131.455	122.610	100.000	92.238	114.897	86.961
2	100.000	117.626	103.144	112.165	100.000	96.854	103.865	86.112
3	100.000	94.790	123.521	116.625	100.000	78.234	80.035	87.219
4	100.000	180.432	159.784	135.414	100.000	100.765	117.064	132.823
5	100.000	174.361	153.193	106.569	100.000	102.280	165.207	175.741
6	100.000	95.065	103.457	88.090	100.000	95.646	99.577	107.985
7	100.000	166.865	141.667	69.444	100.000	71.226	49.057	47.642
8	100.000	100.000	256.857	226.857	100.000	71.575	130.879	71.575
9	100.000	81.352	132.326	90.523	100.000	74.053	73.928	70.765
10	100.000	89.420	100.171	59.727	100.000	224.893	85.837	287.554
11	100.000	97.857	105.664	134.158	100.000	110.821	88.314	75.246
13	100.000	100.000	100.000	100.000	100.000	114.905	94.851	94.851
15	100.000	62.837	62.837	62.837	100.000	98.974	62.308	77.692
17	100.000	174.775	138.559	185.766	100.000	108.197	111.148	108.852
18	100.000	120.341	90.114	145.682	100.000	100.000	100.000	100.000
19	100.000	97.986	89.137	77.093	100.000	109.251	94.728	90.628
20	100.000	110.001	64.865	58.624	100.000	86.649	72.814	85.003
Mean	100.000	117.307	120.985	111.305	100.000	102.151	96.736	105.097
STD	0.000	35.904	44.873	45.530	0.000	34.522	27.274	54.813

Table B19. Percent Change Interleukin-6 (IL-6) %

Subject	Pre Raisin Treatment zero hour	2 hours	3 hours	4 hours	Post Raisin Treatment zero hour	2 hours	3 hours	4 hours
1	100.000	144.587	108.716	108.073	100.000	56.689	80.574	45.413
2	100.000	44.664	82.756	82.756	100.000	85.405	82.797	86.016
3	100.000	70.682	68.258	105.000	100.000	85.485	67.861	99.811
4	100.000	60.619	74.558	86.209	100.000	81.709	128.186	77.361
5	100.000	90.738	100.153	72.366	100.000	110.190	119.429	123.714
6	100.000	80.890	65.131	55.218	100.000	66.784	69.659	67.111
7	100.000	61.557	51.415	88.208	100.000	113.906	95.297	109.611
8	100.000	63.396	89.182	79.119	100.000	76.460	119.161	139.964
9	100.000	49.460	55.093	58.179	100.000	78.988	50.195	93.645
10	100.000	67.183	86.305	89.664	100.000	77.637	93.882	50.211
11	100.000	81.214	81.705	110.531	100.000	92.897	123.189	79.526
13	100.000	130.237	498.221					
15	100.000	76.488	110.317	67.361	100.000	51.877	73.549	79.352
17	100.000	62.007	49.507	54.934	100.000	168.638	65.296	74.550
18	100.000	30.159	39.484		100.000	246.845	91.587	
19	100.000	63.708	49.213	55.056	100.000	68.165	46.942	91.906
20	100.000	100.732	94.005	71.714	100.000	122.508	109.839	162.219
Mean	100.000	75.195	100.236	78.959	100.000	99.012	88.590	92.027
STD	0.000	28.896	104.813	19.206	0.000	48.769	25.881	31.642
Subject	Pre Placebo Treatment zero hour	2 hours	3 hours	4 hours	Post Placebo Treatment zero hour	2 hours	3 hours	4 hours
1	100.000	81.905	79.148	88.120	100.000	180.671	233.912	223.727
2	100.000	116.789	113.394	96.422	100.000	104.180	110.386	137.429
3	100.000	69.298	71.411	94.469	100.000	59.948	66.839	74.677
4	100.000	103.212	101.128	136.892	100.000	84.384	75.526	118.393
5	100.000	63.873	115.378	123.596	100.000	67.406	87.028	115.517
6	100.000	68.160	63.352	65.600	100.000	70.548	80.602	66.623
7	100.000	67.400	82.400	75.400	100.000	51.575	59.843	124.724
8	100.000	74.394	146.765	89.218	100.000	87.595	119.494	262.532
9	100.000	187.239	70.307	96.810	100.000	66.288	61.415	75.167
10	100.000	83.205	314.093	77.606	100.000	63.504	43.750	47.991
11	100.000	135.766	110.949	144.786	100.000	88.739	122.252	126.163
13	100.000	145.213	210.816	1243.085	100.000	81.841	118.285	90.038
15	100.000	65.961	90.879	70.847	100.000	72.544	29.112	107.219
17	100.000	33.678	76.240	51.653	100.000	29.478	34.701	61.567
18	100.000	92.476	67.714	69.905	100.000	46.042	43.333	44.896
19	100.000	83.188	67.904	49.454	100.000	70.790	110.997	180.756
20	100.000	59.211	70.625	86.073	100.000	104.736	91.517	128.897
Mean	100.000	90.057	108.971	156.467	100.000	78.251	87.588	116.842
STD	0.000	37.662	64.634	281.270	0.000	32.787	48.626	59.831

Table B20. Percent Change Soluble Intercellular Adhesion Molecule 1 (sICAM-1) %								
Subject	Pre Raisin Treatment zero hour	2 hours	3 hours	4 hours	Post Raisin Treatment zero hour	2 hours	3 hours	4 hours
1	100.00	89.697	88.132	86.396	100.000	105.672	117.725	119.596
2	100.00	99.063	99.984	96.273	100.000	104.203	108.885	106.105
3	100.00	97.016	95.912	97.556	100.000	95.092	116.649	111.696
4	100.00	92.731	98.304	108.833	100.000	99.913	91.586	90.107
5	100.00	102.457	99.529	107.363	100.000	94.376	95.711	95.425
6	100.00	93.576	107.528	113.013	100.000	72.626	73.330	71.088
7	100.00	102.809	102.901	114.583	100.000	103.562	97.930	102.534
8	100.00	94.926	95.649	110.194	100.000	95.407	92.147	92.472
9	100.00	87.142	99.786	91.836	100.000	94.665	87.736	89.081
10	100.00	102.594	110.036	110.478	100.000	97.936	100.940	103.341
11	100.00	103.756	101.796	94.142	100.000	94.536	92.887	101.312
13	100.00	92.663	87.422		100.000	93.054	90.421	94.596
15	100.00	101.244	94.331	88.743	100.000	89.056	83.836	90.388
17	100.00	93.622	52.358	89.904	100.000	98.983	98.524	100.736
18	100.00	107.649	108.024		100.000	116.764	108.765	
19	100.00	98.962	89.916	91.490	100.000	95.881	98.983	106.888
20	100.00	102.216	87.821	97.849	100.000	153.651	145.383	151.255
Mean	100.00	97.77	95.26	99.91	100.00	100.32	100.08	101.66
STD	0.00	5.66	13.09	9.78	0.00	16.38	16.26	17.24
Subject	Pre Placebo Treatment zero hour	2 hours	3 hours	4 hours	Post Placebo Treatment zero hour	2 hours	3 hours	4 hours
1	100.000	101.810	96.145	99.235	100.000	98.147	103.726	107.188
2	100.000	100.428	95.635	101.625	100.000	106.481	112.865	103.414
3	100.000	95.732	95.470	97.040	100.000	106.178	106.539	101.180
4	100.000	94.155	95.166	105.340	100.000	104.749	91.763	81.124
5	100.000	104.580	108.909	109.848	100.000	103.081	92.436	86.530
6	100.000	99.562	102.196	107.285	100.000	81.229	76.091	80.076
7	100.000	103.113	107.616	110.681	100.000	100.502	99.092	101.306
8	100.000	95.692	102.151	101.941	100.000	90.487	91.922	96.078
9	100.000	93.564	100.301	91.168	100.000	101.270	100.652	97.527
10	100.000	98.947	102.251	104.961	100.000	95.277	96.106	98.885
11	100.000	100.121	88.764	85.942	100.000	93.822	95.937	101.588
13	100.000	91.458	85.056	82.940	100.000	103.958	98.256	102.803
15	100.000	101.490	101.256	98.992	100.000	100.510	100.387	97.825
17	100.000	90.524	91.621	88.186	100.000	103.593	105.010	113.001
18	100.000	102.182	126.413	100.275	100.000	101.610	98.837	103.834
19	100.000	102.468	88.026	84.416	100.000	94.566	103.395	112.585
20	100.000	96.148	94.754	97.963	100.000	98.173	100.797	105.941
Mean	100.00	98.35	98.93	98.11	100.00	99.04	98.46	99.46
STD	0.00	4.27	9.68	8.76	0.00	6.47	7.98	9.39

Table B21. Percent Change Soluble Vascular Cellular Adhesion Molecule 1 (sVCAM-1) %								
Subject	Pre Raisin Treatment zero hour	2 hours	3 hours	4 hours	Post Raisin Treatment zero hour	2 hours	3 hours	4 hours
1	100.000	99.678	100.583	99.182	100.000	98.345	101.776	96.534
2	100.000	98.470	101.249	96.523	100.000	100.765	99.015	100.440
3	100.000	101.307	96.702	98.538	100.000	110.667	113.107	109.031
4	100.000	96.632	100.573	104.599	100.000	96.226	96.042	94.026
5	100.000	98.386	87.380	100.883	100.000	91.986	92.411	98.072
6	100.000	103.581	90.118	93.141	100.000	88.326	91.623	91.452
7	100.000	87.519	81.021	90.733	100.000	105.066	102.056	113.731
8	100.000	91.037	93.650	91.936	100.000	93.141	91.020	98.266
9	100.000	93.822	91.134	88.370	100.000	95.420	95.937	99.578
10	100.000	104.396	104.373	108.238	100.000	100.097	90.560	86.044
11	100.000	98.691	90.336	106.691	100.000	86.303	87.189	85.008
13	100.000	100.636	102.796		100.000	86.292	80.876	76.550
15	100.000	97.842	93.283	95.516	100.000	90.929	86.796	89.010
17	100.000	105.143	104.442	99.232	100.000	89.287	86.795	90.463
18	100.000	103.479	106.180		100.000	109.072	105.544	
19	100.000	105.605	99.306	103.581	100.000	98.622	96.353	101.197
20	100.000	107.112	111.812	95.754	100.000	106.201	102.788	95.998
Mean	100.000	99.608	97.349	98.195	100.000	96.867	95.287	95.337
STD	0.000	5.299	7.878	5.888	0.000	7.722	8.150	9.124
Subject	Pre Placebo Treatment zero hour	2 hours	3 hours	4 hours	Post Placebo Treatment zero hour	2 hours	3 hours	4 hours
1	100.000	102.214	102.611	105.379	100.000	100.734	102.794	100.072
2	100.000	94.549	95.237	97.414	100.000	102.668	99.207	102.457
3	100.000	100.847	105.408	103.852	100.000	103.604	96.724	95.721
4	100.000	101.654	102.258	100.879	100.000	99.681	101.137	94.403
5	100.000	93.662	99.959	102.591	100.000	96.588	97.446	97.020
6	100.000	95.548	87.996	84.973	100.000	93.559	92.506	101.474
7	100.000	84.933	87.632	86.221	100.000	102.041	100.005	106.876
8	100.000	98.146	96.091	99.857	100.000	93.481	92.846	100.228
9	100.000	95.764	97.722	100.169	100.000	100.562	107.554	100.333
10	100.000	99.914	102.450	106.513	100.000	99.429	106.996	116.466
11	100.000	100.397	100.200	103.083	100.000	86.992	85.221	87.093
13	100.000	101.741	100.636	99.509	100.000	115.869	115.315	111.769
15	100.000	97.609	99.446	102.458	100.000	89.086	87.153	88.711
17	100.000	99.705	100.364	98.028	100.000	92.587	96.816	94.856
18	100.000	97.811	99.604	98.711	100.000	105.847	103.816	107.059
19	100.000	106.221	116.478	109.493	100.000	92.713	97.378	91.571
20	100.000	103.527	110.214	103.162	100.000	98.874	98.101	100.271
Mean	100.000	98.485	100.253	100.135	100.000	98.489	98.883	99.787
STD	0.000	4.824	6.906	6.311	0.000	6.919	7.384	7.764

Table B22. Percent Change Free Fatty Acids (FFA) %								
Subject	Pre Raisin Treatment zero hour	2 hours	3 hours	4 hours	Post Raisin Treatment zero hour	2 hours	3 hours	4 hours
1	100.000	40.584	74.536	127.321	100.000	50.524	62.055	103.145
2	100.000	27.157	27.476	76.997	100.000	28.571	27.755	61.429
3	100.000	64.563	68.689	52.184	100.000	18.371	44.089	42.812
4	100.000	9.609	25.623	71.530	100.000	16.912	25.490	41.422
5	100.000	29.387	34.038	29.387	100.000	18.372	28.605	42.093
6	100.000	44.262	90.164	107.650	100.000	34.137	79.116	
7	100.000	65.649	119.847	134.733	100.000	72.112	113.944	139.841
8	100.000	57.312	134.387	217.391	100.000	59.335	109.463	110.742
9	100.000	87.456	88.153	96.864	100.000	70.213	112.340	163.404
10	100.000	171.552	346.552	408.621	100.000	144.253	224.138	308.621
11	100.000	32.746	45.844	78.841	100.000	33.136	93.491	139.053
13	100.000	13.680	21.614		100.000	30.743	34.888	48.187
15	100.000	68.966	85.824	130.651	100.000	31.717	49.899	70.909
17	100.000	218.919	260.811	257.432	100.000	73.333	90.556	105.278
18	100.000	29.040	27.273		100.000	31.618	53.676	
19	100.000	37.920	91.437	100.917	100.000	60.847	185.714	187.302
20	100.000	32.456	41.009	100.000	100.000	38.491	52.830	78.679
Mean	100.000	60.662	93.134	132.701	100.000	47.805	81.650	109.528
STD	0.000	55.260	87.519	96.286	0.000	31.340	55.673	72.010
Subject	Pre Placebo Treatment zero hour	2 hours	3 hours	4 hours	Post Placebo Treatment zero hour	2 hours	3 hours	4 hours
1	100.000	31.187	39.771	58.512	100.000	52.759	46.207	70.172
2	100.000	7.083	16.458	48.750	100.000	16.708	35.661	66.085
3	100.000	8.939	47.486	87.430	100.000	15.776	39.695	71.501
4	100.000	13.291	23.418	41.772	100.000	27.548	21.212	38.292
5	100.000	47.588	64.630	103.215	100.000	20.627	45.953	81.201
6	100.000	65.354	66.929	102.362	100.000	40.341	85.795	131.818
7	100.000	41.444	56.417	71.390	100.000	48.780	98.374	146.341
8	100.000	83.408	119.731	204.484	100.000	50.213	133.617	137.447
9	100.000	39.209	65.827	81.295	100.000	60.177	108.850	91.150
10	100.000	89.333	118.000	143.333	100.000	105.147	126.471	150.000
11	100.000	65.990	116.244	132.487	100.000	34.572	67.658	105.576
13	100.000	44.101	88.202	117.978	100.000	24.349	23.048	33.829
15	100.000	29.787	54.043	74.043	100.000	15.294	28.941	65.647
17	100.000	44.109	65.257	74.622	100.000	81.019	138.889	127.778
18	100.000	47.500	58.125	65.625	100.000	38.743	54.974	91.099
19	100.000	25.676	66.216	79.730	100.000	41.243	95.763	137.006
20	100.000	33.621	50.287	93.103	100.000	23.519	42.549	44.704
Mean	100.000	42.213	65.708	92.949	100.000	40.989	70.215	93.509
STD	0.000	23.576	30.054	39.817	0.000	24.342	40.083	38.975

Table B23. Percent Change Triacylglycerol (TAG) %								
Subject	Pre Raisin Treatment zero hour	2 hours	3 hours	4 hours	Post Raisin Treatment zero hour	2 hours	3 hours	4 hours
1	100.000	112.997	115.291	127.370	100.000	105.790	119.562	130.516
2	100.000	148.334	179.709	182.501	100.000	154.477	168.656	165.671
3	100.000	230.277	245.873	196.331	100.000	117.769	124.793	115.949
4	100.000	108.830	131.886	156.412	100.000	121.955	158.207	199.417
5	100.000	198.976	268.142	251.445	100.000	226.128	250.336	343.987
6	100.000	159.649	176.604	140.291	100.000	123.457	240.148	199.991
7	100.000	210.000	268.499	206.500	100.000	165.979	177.061	173.414
8	100.000	109.473	136.842	182.631	100.000	179.083	197.449	225.064
9	100.000	125.325	164.173	137.455	100.000	162.364	175.092	186.970
10	100.000	194.939	282.268	323.797	100.000	160.080	209.448	238.324
11	100.000	124.487	163.838	163.048	100.000	107.366	148.159	178.754
13	100.000	90.604	105.369		100.000	152.800	155.999	197.598
15	100.000	97.539	184.307	221.229	100.000	185.957	205.531	273.615
17	100.000	208.235	221.764	211.176	100.000	207.999	210.998	212.999
18	100.000	124.998	81.667		100.000	116.333	94.814	
19	100.000	139.909	151.248	164.399	100.000	124.490	146.754	145.640
20	100.000	165.102	181.209	181.880	100.000	146.860	188.889	213.527
Mean	100.000	149.981	179.923	189.764	100.000	150.523	174.817	200.090
STD	0.000	44.124	59.748	50.181	0.000	35.291	42.027	55.354
Subject	Pre Placebo Treatment zero hour	2 hours	3 hours	4 hours	Post Placebo Treatment zero hour	2 hours	3 hours	4 hours
1	100.000	107.769	130.827	130.075	100.000	107.792	141.248	142.641
2	100.000	63.704	67.408	67.408	100.000	141.096	98.501	149.314
3	100.000	196.994	239.026	276.209	100.000	111.451	112.988	103.223
4	100.000	41.823	58.087	99.374	100.000	142.767	155.678	152.450
5	100.000	154.537	184.912	65.843	100.000	154.531	182.353	248.013
6	100.000	43.118	141.665	174.999	100.000	175.360	357.374	444.671
7	100.000	135.206	185.018	153.932	100.000	129.147	179.154	178.176
8	100.000	179.243	221.731	249.555	100.000	132.400	161.648	188.757
9	100.000	192.884	201.187	150.853	100.000	166.596	207.539	172.516
10	100.000	177.513	232.965	227.599	100.000	141.358	173.368	178.110
11	100.000	91.497	94.824	108.503	100.000	133.233	133.233	132.629
13	100.000	132.314	157.409	125.863	100.000	120.755	116.981	137.736
15	100.000	131.501	210.622	265.566	100.000	135.205	156.928	235.580
17	100.000	181.651	187.309	203.045	100.000	171.349	199.439	201.686
18	100.000	106.061	138.042	109.522	100.000	124.196	109.678	114.518
19	100.000	148.411	190.465	164.059	100.000	120.937	182.095	191.870
20	100.000	164.248	217.099	241.969	100.000	137.075	140.816	146.130
Mean	100.000	132.263	168.153	165.551	100.000	137.956	165.236	183.413
STD	0.000	50.077	55.662	68.018	0.000	19.653	58.992	77.771

Table B24. Percent Change Glucose %

Subject	Pre Raisin Treatment zero hour	2 hours	3 hours	4 hours	Post Raisin Treatment zero hour	2 hours	3 hours	4 hours
1	100.00	76.49	80.17	109.92	100.00	117.45	121.48	133.56
2	100.00	94.03	96.19	97.35	100.00	106.90	104.21	101.85
3	100.00	110.88	119.49	101.36	100.00	100.99	103.96	100.99
4	100.00	85.90	96.77	92.36	100.00	96.96	102.43	103.34
5	100.00	121.70	123.43	103.14	100.00	121.13	102.99	99.82
6	100.00	115.56	106.42	109.92	100.00	102.98	105.78	99.12
7	100.00	87.29	107.53	101.51	100.00	108.12	121.40	120.30
8	100.00	84.76	101.98	115.70	100.00	110.27	106.02	115.93
9	100.00	127.90	128.96	126.09	100.00	121.22	118.26	128.45
10	100.00	109.34	126.93	140.22	100.00	133.64	119.85	129.60
11	100.00	117.57	102.95	103.58	100.00	96.21	100.36	107.22
13	100.00	113.33	103.86		100.00	110.58	107.12	105.66
15	100.00	102.94	114.51	116.58	100.00	104.95	128.45	120.49
17	100.00	100.82	117.70	121.97	100.00	107.12	117.29	128.14
18	100.00	79.71	78.40		100.00	65.21	84.54	
19	100.00	114.77	118.94	107.20	100.00	93.20	105.19	100.72
20	100.00	117.29	79.28	88.01	100.00	102.09	111.24	110.43
Mean	100.00	103.55	106.09	108.99	100.00	105.82	109.44	112.85
STD	0.00	15.99	16.17	13.56	0.00	14.70	10.60	12.26
Subject	Pre Placebo Treatment zero hour	2 hours	3 hours	4 hours	Post Placebo Treatment zero hour	2 hours	3 hours	4 hours
1	100.00	68.79	86.13	80.35	100.00	140.73	100.61	105.17
2	100.00	103.32	95.02	98.01	100.00	82.79	96.93	96.93
3	100.00	80.59	79.41	89.04	100.00	96.88	109.20	100.17
4	100.00	90.79	101.78	91.53	100.00	87.18	101.46	105.68
5	100.00	107.64	97.28	95.93	100.00	87.30	91.36	99.82
6	100.00	101.85	107.39	92.27	100.00	116.83	120.94	111.55
7	100.00	106.13	105.32	93.55	100.00	99.44	115.38	117.82
8	100.00	101.48	101.67	134.26	100.00	99.12	103.35	120.11
9	100.00	88.33	87.44	93.80	100.00	114.51	107.96	100.16
10	100.00	100.17	103.46	102.77	100.00	118.38	108.83	119.82
11	100.00	96.12	98.76	94.11	100.00	106.48	103.24	98.81
13	100.00	88.11	105.24	97.90	100.00	118.00	106.85	113.70
15	100.00	121.63	112.01	129.21	100.00	115.26	98.92	120.11
17	100.00	109.23	95.99	119.51	100.00	97.99	123.63	124.18
18	100.00	88.73	95.17	109.84	100.00	102.97	101.22	100.52
19	100.00	100.37	105.40	128.12	100.00	96.69	96.86	106.10
20	100.00	144.33	132.30	108.42	100.00	106.79	104.88	102.96
Mean	100.00	99.86	100.58	103.45	100.00	105.14	105.39	108.45
STD	0.00	16.74	11.69	15.71	0.00	14.46	8.50	9.11

Table B25. Percent Change Insulin %

Subject	Pre Raisin Treatment zero hour	2 hours	3 hours	4 hours	Post Raisin Treatment zero hour	2 hours	3 hours	4 hours
1	100.000	420.117	191.417	175.933	100.000	398.617	233.450	271.376
2	100.000	357.872	264.972	153.563	100.000	553.883	494.717	226.767
3	100.000	349.939	649.848	335.374	100.000	389.453	259.785	264.938
4	100.000	513.465	293.166	174.975	100.000	414.500	330.100	325.683
5	100.000	676.562	714.311	355.274	100.000	1065.210	362.916	238.170
6	100.000	286.383	181.850	100.000	100.000	281.417	117.127	100.000
7	100.000	223.683	183.280	86.937	100.000	437.861	212.140	169.183
8	100.000	250.017	179.767	137.300	100.000	109.690	130.692	127.852
9	100.000	500.964	404.487	306.738	100.000	507.322	358.322	390.320
10	100.000	392.265	308.359	134.372	100.000	495.983	213.483	150.175
11	100.000	466.156	269.006	229.445	100.000	144.213	95.973	78.716
13	100.000	921.517	342.800		100.000	717.436	418.751	265.429
15	100.000	476.067	301.550	152.348	100.000	226.602	340.824	69.932
17	100.000	359.383	368.083	387.833	100.000	311.883	455.850	373.800
18	100.000	324.433	184.683		100.000	100.000	147.913	
19	100.000	226.167	135.365	100.000	100.000	134.273	161.065	203.750
20	100.000	530.786	202.809	153.190	100.000	403.781	459.354	158.673
Mean	100.000	427.987	304.456	198.885	100.000	393.655	281.910	213.423
STD	0.000	175.766	161.454	99.572	0.000	243.795	130.740	99.027
Subject	Pre Placebo Treatment zero hour	2 hours	3 hours	4 hours	Post Placebo Treatment zero hour	2 hours	3 hours	4 hours
1	100.000	299.224	299.639	134.568	100.000	468.850	384.117	326.078
2	100.000	348.151	134.922	90.528	100.000	242.468	146.164	80.630
3	100.000	656.836	321.638	185.228	100.000	289.920	302.564	100.431
4	100.000	342.203	224.688	142.756	100.000	444.027	361.707	257.713
5	100.000	694.046	398.218	180.365	100.000	1122.083	435.717	287.667
6	100.000	265.683	366.533	100.000	100.000	306.433	239.400	118.478
7	100.000	368.698	189.245	78.734	100.000	233.764	149.757	
8	100.000	104.287	100.000	100.000	100.000	145.331	101.152	102.280
9	100.000	569.000	263.106	191.333	100.000	562.974	543.922	183.191
10	100.000	248.611	219.059	127.735	100.000	525.733	239.367	142.395
11	100.000	221.186	115.748	124.604	100.000	284.922	157.594	89.318
13	100.000	357.275	254.140		100.000	606.717	482.217	228.433
15	100.000	312.967	129.132	116.565	100.000	374.183	132.173	182.783
17	100.000	493.550	370.967	389.850	100.000	233.600	416.150	390.083
18	100.000	214.383	382.217	171.250	100.000	265.400	118.315	137.257
19	100.000	208.300	100.000	365.500	100.000	168.367	135.228	215.617
20	100.000	856.701	856.701	260.776	100.000	318.935	237.167	157.580
Mean	100.000	385.947	277.997	172.487	100.000	387.865	269.571	187.496
STD	0.000	202.038	181.379	92.606	0.000	233.024	142.618	90.861

Appendix C

Statistical Analyses of Lab Measurements

STATISTICAL PROCEDURES:

Descriptive statistics were used to summarize the data. Baseline student's t-tests were used to identify differences between genders for initial subject characteristics and measures. Repeated measures (RM) ANOVA were used for fasting (times were pre and post) and postprandial (times were 0, 2, 3, 4 h) data. In order to examine postprandial changes in relation to the fasted values for the factor at time 0, each measurement was expressed as a percent change of the initial time 0 value (100%) and a 3 factor mixed model ANOVA was used to determine the main effects of either treatment (raisin or placebo), time (2, 3, 4 hours postprandial), intervention (pre and post), gender (male or female), order (period), and the interaction among these main factors. All analyses were computed via SAS software (version 9.1.3; SAS Institute Inc., Cary, N.C.). A mixed model was used in order to circumvent case-wise deletion of subjects with missing data points. Post hoc analyses were done using the Tukey-Kramer test for significance to identify the source of significant effects. Pearson correlation coefficients were performed for screening blood draw (fasted) actual values (not % change) and measures of obesity; hip to waist ratio, waist circumference and BMI measures as well as metabolite change scores. Change scores were calculated as peak postprandial response less time 0 for all 4 meal challenge. Statistical results for all measures except glucose, sICAM-1, and sVCAM-1 were based on log (ln) or square root-transformation of the percent change data: analyses for CRP, IL-6, ORAC_{total}, ORAC_{pca}, 8-epi and insulin used the log response, the square root response for FFA, and triacylglycerol. Log and square root transformations were done in order to accommodate nonnormal distributions of the data. Insulin RM ANOVA fasted comparisons were based on differences computed by subtracting post from pre treatment values and the significance was based on the difference from 0. A separate analysis was required for insulin since ANOVA assumptions were not upheld under the standard analysis due to a high number of identical values. CI was computed for log-transformed data requiring back transformation in order to obtain group means expressed as actual values. Fasting data was expressed as mean and 95% confidence interval (CI), and data for initial subject characteristics and diet analysis were expressed as mean $\pm$ SD unless otherwise stated. A p value of <0.05 was considered significant.

Definitions of Fixed Effects:

Gender: Male or Female

Treatment: Raisin or Jelly Candy

Period: The two four week periods separated by the crossover

Prepost: Pre and Post Treatment

Time: Postprandial Observations

Table C26. RMANOVA Fasting Urinary 8-iso-Prostagandin F2α				
Effect	Num DF	Den DF	F value	Pr > F
Gender	1	15.2	0.00	0.9777
Treatment	1	14.4	1.28	0.2761
Period	1	14.4	0.86	0.3680
Prepost	1	31	18.30	0.0002
Gender*Prepost	1	31	2.92	0.0973

Table C27. RMANOVA Fasting Serum Total Oxygen Radical Antioxidant Capacity (ORACtotal)				
Effect	Num DF	Den DF	F value	Pr > F
Gender	1	14	0.97	0.3408
Treatment	1	15	16.08	0.0011
Period	1	15	0.00	0.9990
Treatment*Period	1	14	0.49	0.4953
Prepost	1	29	5.24	0.0296
Gender*Prepost	1	29	2.54	0.1220
Treatment*Prepost	1	29	3.19	0.0847
Period*Prepost	1	29	0.11	0.7446
Treatment*Period*Prepost	1	29	9.72	0.0041

Table C28. RMANOVA Fasting Serum Oxygen Radical Antioxidant Capacity (ORACpca) perchloric acid (PCA)				
Effect	Num DF	Den DF	F value	Pr > F
Gender	1	14	0.79	0.3877
Treatment	1	14	15.34	0.0016
Gender*Treatment	1	14	4.87	0.0445
Period	1	14	0.27	0.6096
Treatment*Period	1	14	2.65	0.1257
Prepost	1	32	10.29	0.0030
Treatment*Prepost	1	32	0.36	0.5506

Table C29. RMANOVA Fasting Serum C-Reactive Protein (CRP)				
Effect	Num DF	Den DF	F value	Pr > F
Gender	1	14	2.88	0.1119
Treatment	1	15	0.02	0.8776
Period	1	15	0.51	0.4856
Treatment*Period	1	15	1.08	0.3155
Prepost	1	29	0.00	0.9950
Treatment*Prepost	1	29	0.06	0.8141
Period*Prepost	1	29	1.73	0.1992
Treatment*Period*Prepost	1	29	6.14	0.0193

Table C30. RMANOVA Fasting Serum Interleukin-6 (IL-6)				
Effect	Num DF	Den DF	F value	Pr > F
Gender	1	14	1.11	0.3109
Treatment	1	15	0.16	0.6944
Period	1	15	1.85	0.1939
Treatment*Period	1	15	2.12	0.1660
Prepost	1	29	50.70	0.0321
Treatment*Prepost	1	29	0.16	0.6879
Period*Prepost	1	29	0.39	0.5352
Treatment*Period*Prepost	1	29	3.78	0.0615

Table C31. RMANOVA Fasting Serum Soluble Intercellular Adhesion Molecule 1 (sICAM-1)				
Effect	Num DF	Den DF	F value	Pr > F
Gender	1	15	1.11	0.3079
Treatment	1	15	1.35	0.2643
Period	1	15	0.53	0.4791
Prepost	1	32	1.17	0.2881
Treatment*Prepost	1	32	2.26	0.1423

Table C32. RMANOVA Fasting Serum Soluble Vascular Cellular Adhesion Molecule 1 (sVCAM-1)				
Effect	Num DF	Den DF	F value	Pr > F
Gender	1	14	0.39	0.5416
Treatment	1	15	0.05	0.8346
Period	1	15	0.00	0.9854
Treatment*Period	1	15	0.00	0.9940
Prepost	1	32	6.45	0.0162
Treatment*Prepost	1	32	3.22	0.0822

Table C33. RMANOVA Fasting Serum Free Fatty Acids (FFA)				
Effect	Num DF	Den DF	F value	Pr > F
Gender	1	15	3.39	0.0854
Treatment	1	15	11.34	0.0042
Period	1	15	4.70	0.0467
Prepost	1	32	3.70	0.0634
Treatment*Prepost	1	32	11.51	0.0019

Table C34. RMANOVA Fasting Serum Triacylglycerol (TAG)				
Effect	Num DF	Den DF	F value	Pr > F
Gender	1	15	1.62	0.2223
Treatment	1	15	0.26	0.6155
Period	1	15	0.55	0.4704
Prepost	1	33	0.16	0.6919

Table C35. RMANOVA Fasting Serum Glucose				
Effect	Num DF	Den DF	F value	Pr > F
Gender	1	14	0.01	0.9348
Treatment	1	14	1.27	0.2792
Gender*Treatment	1	14	4.68	0.0482
Period	1	14	6.87	0.0201
Prepost	1	33	22.53	<.0001

Table 36. RMANOVA Serum Insulin				
Effect	Num DF	Den DF	F value	Pr > F
Gender	1	15	0.64	0.4364
Treatment	1	15	0.29	0.5979
Period	1	15	3.10	0.0987

Table C37. RMANOVA Percent Change C-Reactive Protein (CRP) %				
Effect	Num DF	Den DF	F value	Pr > F
Gender	1	14	0.22	0.6430
Treatment	1	15	0.28	0.6029
Period	1	15	0.65	0.4312
Treatment*Period	1	15	0.79	0.3875
Prepost	1	32	2.51	0.1226
Time	2	123	0.4	0.6683
Treatment*Time	2	123	0.85	0.4306
Period*Time	2	123	0.66	0.5162
Treatment*Period*Time	2	123	3.22	0.0435

Table C38. RMANOVA Percent Change Interleukin-6 (IL-6) %				
Effect	Num DF	Den DF	F value	Pr > F
Gender	1	15	2.34	0.1468
Treatment	1	15	0.01	0.9185
Period	1	15	0.51	0.4850
Prepost	1	31	0.32	0.5774
Period*Prepost	1	31	3.10	0.0882
Time	2	129	2.97	0.0546

Table 39. Percent Change Soluble Intercellular Adhesion Molecule-1 (sICAM-1) %				
Effect	Num DF	Den DF	F value	Pr > F
Gender	1	14	1.70	0.2132
Treatment	1	14	0.13	0.7215
Gender*Treatment	1	14	2.46	0.1390
Period	1	14	1.53	0.2365
Prepost	1	32	0.53	0.4704
Gender*Prepost	1	32	2.90	0.0985
Time	2	131	1.46	0.2350

Table C40. RMANOVA Percent Change Soluble Vascular Cellular Adhesion Molecule-1 (sVCAM-1) %				
Effect	Num DF	Den DF	F value	Pr > F
Gender	1	14	3.88	0.0688
Treatment	1	15	0.08	0.7870
Period	1	15	1.85	0.1937
Treatment*Period	1	15	0.29	0.6005
Prepost	1	33	1.12	0.2981
Time	2	123	2.16	0.1198
Gender*Time	2	123	3.86	0.0237
Treatment*Time	2	123	1.29	0.2786
Period*Time	2	123	3.55	0.0317
Treatment*Period*Time	2	123	3.05	0.0512

Table. C41 RMANOVA Percent Change Free Fatty Acids (FFA) %				
Effect	Num DF	Den DF	F value	Pr > F
Gender	1	14	15.00	0.0017
Treatment	1	15	9.69	0.0071
Period	1	15	2.32	0.1487
Treatment*Period	1	15	1.86	0.1923
Prepost	1	32	0.63	0.4318
Treatment*Prepost	1	32	6.37	0.0168
Time	2	122	149.55	<.0001
Gender*Time	2	122	5.03	0.0079
Treatment*Time	2	122	2.85	0.0616
Period*Time	2	122	0.14	0.8729
Treatment*Period*Time	2	122	6.26	0.0026

Table C42. RMANOVA Percent Change Triacylglycerol (TAG) %				
Effect	Num DF	Den DF	F value	Pr > F
Gender	1	14	5.56	0.0334
Treatment	1	14	0.95	0.3453
Period	1	14	1.53	0.2368
Gender*Period	1	14	2.13	0.1668
Prepost	1	33	0.51	0.4804
Time	2	127	31.82	<.0001
Gender*Time	2	127	4.68	0.0109
Prepost*Time	2	127	3.07	0.0500

Table C43. RMANOVA Percent Change Glucose %				
Effect	Num DF	Den DF	F value	Pr > F
Gender	1	14	10.26	0.0064
Treatment	1	14	0.55	0.4712
Gender*Treatment	1	14	2.19	0.1610
Period	1	14	0.00	0.9543
Prepost	1	30	4.97	0.0334
Gender*Prepost	2	30	6.59	0.0155
Treatment*Prepost	1	30	0.65	0.4278
Gender*Treatment*Prepost	1	30	6.74	0.0145
Time	2	127	0.02	0.0521
Gender*Time	2	127	3.50	0.0331
Treatment*Time	2	127	3.13	0.0473

Table C44. RMANOVA Percent Change Insulin %				
Effect	Num DF	Den DF	F value	Pr > F
Gender	1	13	0.15	0.7041
Treatment	1	13	0.35	0.5647
Gender*Treatment	1	13	2.40	0.1454
Period	1	13	1.76	0.2074
Gender*Period	1	13	0.02	0.8867
Prepost	1	29	0.14	0.7106
Gender*Prepost	1	30	1.86	0.1834
Treatment*Prepost	1	30	10.23	0.0033
Period*Prepost	1	30	8.51	0.0067
Gender*Period*Prepost	1	30	5.92	0.0214
Time	2	131	46.65	<.0001

Table C45. Paired t-test for Initial Subject Characteristics			
	T	DF	P
Age (years)	2.263	7	0.058
Weight (kg)	1.770	7	0.120
BMI (kg/m ²)	-1.864	7	0.105
Waist Circumference (cm)	0.257	7	0.805
Hip to Waist Ratio (WHR)	2.178	7	0.066
Free Fatty Acids (μmol/L)	-0.409	6	0.696
Triacylglycerol (mg/dL)	1.139	7	0.292
Glucose (mg/dL)	-0.599	7	0.568
Insulin (μIU/mL) [†]	-2.461	7	0.043
CRP (mg/L)	-2.255	6	0.065
IL-6 (pg/mL)	-0.380	7	0.715
sICAM-1 (ng/mL)	-1.887	7	0.101
sVCAM-1 (ng/mL)	0.500	7	0.632
ORAC _{total} (μmol/L TE)	-0.261	7	0.801
ORAC _{pca} (μmol/L TE)	0.656	7	0.533
Urinary 8-epi PGF _{2a} (pg/mg Cr) [*]	-0.787	7	0.457

^{*}CR, creatinine

Appendix D

Health History Questionnaire and Supplemental Questions

MEDICAL AND HEALTH HISTORY

Name: _____ Age: _____ Birth Date: _____

Address: _____ e-mail: _____

Phone Numbers: Home: _____ Work : _____

Summer Address: _____

Phone Number: _____

Person to Contact in Case of an Emergency: _____

Relationship: _____ Phone: _____

Primary Care Physician: _____ Phone: _____

Medical Insurance Carrier: _____

Are you employed by Virginia Tech? _____

Current Body Weight: _____ Height _____

MEDICAL HISTORY

Please indicate any current or previous conditions or problems you have experienced or have been told by a physician you have had:

	Yes	No
Heart disease or any heart problems:	_____	_____
Rheumatic Fever:	_____	_____
Respiratory disease or breathing problems (e.g. asthma):	_____	_____
Circulation problems:	_____	_____
Kidney disease or problems:	_____	_____
Urinary problems:	_____	_____
Musculoskeletal problems: (i.e. Orthopedic injuries, osteoporosis)	_____	_____
Fainting and Dizziness:	_____	_____
High Cholesterol:	_____	_____
Diabetes:	_____	_____
Thyroid problems:	_____	_____
Mental illness:	_____	_____
Hypoglycemia:(i.e. low blood sugar)	_____	_____
Epilepsy or seizures:	_____	_____
Blood clotting problems (e.g. hemophilia):	_____	_____
Liver disorders (e.g. hepatitis B)	_____	_____
Rheumatoid arthritis	_____	_____
Lupus	_____	_____

Crohn's Disease _____

If you answered "yes" to any of the previous questions, please indicate the date and describe:

Please list any hospitalizations/operations/recent illnesses (type/date):

	Yes	No
Have you ever been diagnosed as having high blood pressure?	_____	_____

Are you currently being treated for high blood pressure?	_____	_____
--	-------	-------

If "yes", please explain:

Please list all medications (prescription and over-the-counter) you are currently taking or have taken in the past week: _____

For what reason(s) are you taking this medication?

Health Habits

	Yes	No
Do you drink alcoholic beverages?	_____	_____
How many drinks per week? _____		
Do you smoke cigarettes?	_____	_____
Packs per day: _____		

Do you engage in regular exercise?	Yes _____	No _____
------------------------------------	--------------	-------------

If "yes", please list:

Activity	Frequency (times per week)	Duration (minutes)
_____	_____	_____
_____	_____	_____

Do you ever faint, experience shortness of breath or chest discomfort with exertion? _____

If “yes”, please explain: _____

Are there any orthopedic limitations you have that may restrict your ability to perform exercise and if “yes”, please explain:

Family History

Has anyone in your family been diagnosed or treated for any of the following?

	Yes	No	Relationship	Age
Heart attack	_____	_____	_____	_____
Heart disease	_____	_____	_____	_____
High blood pressure	_____	_____	_____	_____
Stroke	_____	_____	_____	_____
Kidney disease	_____	_____	_____	_____
Diabetes	_____	_____	_____	_____

Schedule Spring 2005 – Summer I 2005 semester (indicate those times you have classes, work etc that you CANNOT be involved in study activities):

	<i>Mon</i>	<i>Tue</i>	<i>Wed</i>	<i>Thursday</i>	<i>Fri</i>
6:00-7:00am					
7:00-8:00					
8:00-9:00					
9:00-10:00					
10:00-11:00					
11:00-12:00					
12:00-1:00					
1:00-2:00					
2:00-3:00					
3:00-4:00					
4:00-5:00					
5:00-6:00					
6:00-7:00					
7:00-8:00					

Any explanation required for above _____

Please sign to indicate that the above information is correct:

_____	_____	_____
Print name	Signature	Date

SUPPLEMENTARY QUESTIONS

Food Habits and Allergies

1. Are you allergic to any foods? ____ If yes, which ones?
2. Are you on any kind of special diet? ____ If so, what kind?
3. Do you take any dietary supplements? ____ If so, what kind and how often?
4. Has your weight been stable over the past year? ____ past 3 months? If not, how has it changed?

Comfort with procedures

5. Do you have a fear of needles or having blood withdrawn?

Appendix E
Infection Questionnaire

INFECTION/INFLAMMATION QUESTIONNAIRE

Evaluator Script: I would like you to think if you had a cold, the flu, a dental infection or other infection during the past month. I am going to ask you about some symptoms that may have accompanied those types of conditions.

1) Did you have a cold, the flu, a dental infection or other infection in the past month?

☐ Yes ☐ No ☐ Refused ☐ Don't Know

If yes, ☐ Within 1 week ☐ 2 weeks prior ☐ 3 weeks prior ☐ 4 weeks prior

In the prior month did you experience any of the following symptoms? [Note to examiner: If symptom was present, the timing of symptom onset and resolution (# days) prior to interview is recorded. If symptom is still present on the day of interview, place 0 in "Resolved___days ago".]

2) Did you feel feverish or have a fever? ☐ Yes ☐ No

If Yes, Symptom Started ___days ago. Resolved___days ago.

Did you take your temperature? ☐ Yes ☐ No

3) Chills? ☐ Yes ☐ No

If Yes, Started___days ago. Resolved___days ago.

4) Sore throat ? ☐ Yes ☐ No

If Yes,.Started___days ago. Resolved___days ago.

5) Coughing? ☐ Yes ☐ No

If Yes, Started___days ago. Resolved___days ago.

6) Sputum? ☐ Yes ☐ No

If Yes,.Started___days ago. Resolved___days ago.

7) Sneezing? ☐ Yes ☐ No

If Yes, Started___days ago. Resolved___days ago.

8) Runny nose or nasal congestion? ☐ Yes ☐ No

If Yes, Started___days ago. Resolved___days ago.

If Yes to (5), (6), (7), or (8). Do you have seasonal allergies? ☐ Yes ☐ No

Do you have a chronic lung or sinus condition? ☐ Yes ☐ No

If Yes, are these symptoms typical for your chronic lung or sinus condition?

☐ Yes ☐ No

9) Ear pain or discharge? ☐ Yes ☐ No

If Yes, Started____days ago. Resolved____days ago.

10) Run down feeling or achy muscles you feel may have been due to a cold or flu?

☐ Yes ☐ No

If Yes, Started____days ago. Resolved____days ago.

11) Tooth/Gum pain? ☐ Yes ☐ No

If Yes, Started____days ago. Resolved____days ago.

If Yes, did you seek dental care?(☐ Yes ☐ No

If Yes, did a Dentist find a cavity or other dental infection? ☐ Yes ☐ No

12) Mouth/gum (Y N), Skin (Y N), or Joint (Y N) redness or swelling?

If Yes, Started____days ago. Resolved____days ago.

13) Skin infection? ☐ Yes ☐ No

If Yes, Started____days ago. Resolved____days ago.

14) Nausea/Vomiting? ☐ Yes(☐ No

If Yes, Started____days ago. Resolved____days ago.

15) Diarrhea? ☐ Yes ☐ No

If Yes, Started____days ago. Resolved____days ago.

16) Pain upon urination or urgency? ☐ Yes ☐ No

If Yes, Started____days ago. Resolved____days ago.

17) Cloudy discolored urine? ☐ Yes ☐ No

Urinalysis showing evidence of infection? ☐ Yes ☐ No

If Yes, Started____days ago. Resolved____days ago.

18) Did you seek medical care for any sort of cold, flu, or infection in the prior month?

(☐)Yes (☐)No

If yes, diagnosis given_____

19) Did you take any over the counter or prescription medications for a cold, flu, or any infection in the prior month?

(☐)Yes (☐)No

If yes, names of medication_____

Appendix F
Institutional Review Board Request

Protocol for Investigation Involving Human Subjects

Project Title: Value of Raisins for Reduction of Oxidative Stress, Endothelial Dysfunction, and Inflammation in Obesity.

Investigators:

PI: Janet W. Rankin, Ph.D (HNFE)

Co-Pis: Brenda Davy, R.D., Ph.D (HNFE), Kevin Davy Ph.D. (HNFE), Sean O'Keefe, Ph.D. (FST)

Students: Abigail D. Turpyn, B.S. (doctoral candidate), Mary Whitlock, B.S. (Master's candidate)

Justification / Purpose of Project

Data from the third National Health and Nutrition Examination Survey (NHANESIII) demonstrate that over 64% of adults in the US are overweight and about 30% obese, (Flegal et al 2002). This increases risk of cardiovascular disease and some cancers in these individuals. Part of the mechanism for this association may be elevated blood lipids or blood pressure but some research suggests that the higher oxidative stress in overweight individuals triggers an inflammatory response that increases disease risk. Numerous studies support the positive relationship between body mass index (BMI) and indicators of oxidative stress. For example, 8-isoPGF_{2α} has been shown consistently higher in obese than lean individuals (Keaney, 2003; Desideri, 2003; Davi, 2002). This member of the isoprostane family is a biomarker of oxidative stress (Roberts, 1997). Keaney et al (2003), using a large cohort that included 2828 subjects from the Framingham Heart Study, found a direct positive correlation between urinary creatinine-indexed 8-epi PGF_{2α} excretion and BMI. In fact, with each 5 kg/m² of extra weight there was an associated 9.9% increase in urinary creatinine-indexed 8-epi PGF_{2α} level.

Obese individuals commonly have elevated serum inflammatory markers such as C-reactive protein (CRP) and cytokines such as IL-6 (Das, 2001; Esposito, 2002; Hak, 1999; Mohammed-Ali, 1997; Visser, 1999, Yudkin, 1999). CRP has been identified as an independent predictor of future cardiovascular events (Wanner, 2002). Some research links oxidative stress to inflammation in obese humans. Lipid peroxidation, 8-iso PGF_{2α} levels, and platelet activation (a process associated with inflammation and measured by measured by 11-dehydrothromboxane B2 levels) were positively associated with the higher quartiles of CRP levels in women with obesity (Davi et al 2002). An earlier study by the same research group found a statistically significant correlation between the rates of 8-epi-PGF_{2α} excretion and 11-dehydro-thromboxane B2 (Davi, 1997). This indicates a potential link between lipid peroxidation and inflammation

Oxidative stress has also been linked to blood vessel dysfunction, suggesting increased risk of cardiovascular disease. Flow-mediated brachial artery vasoactivity (FMV), used as a measure of blood vessel function, has been associated with oxidative stress. Several studies have shown that consumption of a high fat meal in normal weight individuals (Lee et al 2002, Vogel et al 1997) impairs endothelial function. Lee et al (2002) verified that the reduction in FMV was concurrent with elevated oxidative stress. The role of oxidative stress in this process was more strongly supported by the fact that vitamin E prevented the drop in FMV from the high fat meal.

Thus, a target of obesity treatment could be reduction in oxidative stress which is hypothesized to subsequently reduce inflammation as well as endothelial dysfunction. Although weight loss clearly reduces oxidative stress in obese individuals (Davi 2002, Desideri 2003), few

people successfully lose and maintain weight lost. Thus, it would be useful to have simple, easy- to- follow dietary approaches that reduce oxidative stress and thus reduces disease risk factors.

Some dietary components have been examined for their effect on oxidative stress. Chronic consumption of fruits and vegetables has been associated with lower basal markers of oxidative stress (Thompson et al 1999). Other studies have examined the effect of dietary modification on response to an oxidative stressor. For example, in a study of 12 obese and 12 lean subjects, the effect of 3 different diets (DASH combination diet, a low antioxidant diet, control ad libitum diet) over 4 weeks on oxidative stress response to a meal challenge was measured (Lopes, 2003). One of the hallmarks of the DASH combination diet is its provision of substantial servings of fruits and vegetables. A test was performed to artificially increase serum fatty acids as would happen with a high fat meal following each dietary intervention to assess changes in markers of oxidative stress. Markers of oxidative stress increased with test in subjects on the low antioxidant diet but not in those who consumed the DASH combination diet suggesting that the diet was protective.

Plotnick et al (2003) observed that the reduction in FMV following a high fat meal was prevented by consumption of a high antioxidant supplement made from vegetables and fruits. Esposito et al (2003) did a similar acute meal challenge (760 kcal, 59.2% fat, 28.5% CHO, and 12.3% protein), in normal, healthy subjects and examined endothelial function. The high fat meal impaired endothelial function but when the meal was supplemented with antioxidants using various vegetables (100g tomatoes, 200g carrots, and 100g green peppers which provided a total of 184 mg Vitamin C, 19.65mg E, 15mg β -carotene, and 9.2g fiber), the vascular response returned to baseline.

Thus, there is evidence that consumption of foods or supplements high in antioxidants can blunt or prevent the acute reduction in FMV following a high fat meal. This is likely mediated through reduction in oxidative stress. Reduction in oxidative stress, inflammation, and endothelial dysfunction response to a high fat meal would be expected to reduce risk of heart disease in overweight individuals.

Raisins, like many other fruits, are a good source of flavonoids and phenolic acids, compounds with antioxidant effects. The phenolic content of raisins is likely a major factor that could contribute to antioxidant and health-promoting abilities. Karakaya et al (2001) measured the total phenol content of 11 beverages and 13 solid foods including grapes and raisins. Raisins contained 3994 ± 576 mg/kg total phenols compared to 1580 ± 371 mg/kg for green and 2206 ± 612 mg/kg for red grapes. Although no studies have been published on antioxidant effects of raisin ingestion, several studies have tested the value of grapes or grape juice as a dietary intervention. O'Byrne et al (2002) found that 10 mg/kg concord grape juice was as effective as 400 IU vitamin E in reducing some markers of oxidative stress (e.g. urinary F2 isoprostanes) and more effective in others (plasma protein carbonyl concentration). The grape juice consumed each day was measured to contain 560 mg phenolic equivalents. Other studies (Freedman et al 2001, Stein et al 1999) found benefits with even smaller doses of grape juice (~7 ml/kg or approximately 392 mg phenolic equivalents). Thus, using the measurements of Karakaya et al (2001), the 90 g of raisins (equivalent to 2 fruit servings per day) we propose in our study below would have an estimated 359 mg of phenolic equivalents, an amount likely to have beneficial antioxidant effects. In addition, it is a quantity that could logically be consumed by free- living individuals.

Thus, the hypothesis tested in this study is that:
Regular consumption of raisins will reduce the oxidative stress, endothelial dysfunction, and inflammation response to a high fat meal challenge in overweight individuals compared to an isoenergetic carbohydrate snack.

Procedures

Twenty overweight men or women will be recruited from the local area. The subjects will be recruited via word of mouth, advertisements, flyers, and email notifications. The flyers will contain a brief description of the study and the contact information, including a phone number and email address (see Appendix 1).

Initial selection criteria will include self-reported age (18-50 yr), low level of physical activity level (≤ 60 minutes of planned moderate to high intensity exercise per week), body mass index $> 27 \text{ kg/m}^2$, stable body weight ($\pm 0.5 \text{ kg}$ over past 3 months), and abdominal obesity (waist circumference $> 102 \text{ cm}$ for men, $> 88 \text{ cm}$ women). Exclusion criteria will include cigarette smoking as well as conditions known to affect inflammation and oxidative stress (e.g., Crohn's disease, diabetes).

Interested participants will be asked to contact the investigators via e-mail or telephone to obtain a general description of the study. A screening form will be administered via email or by mail, if the participants do not have access to email. The screening form will include questions about their height and weight (to determine BMI), weight history, medical history, current medication use, and exercise history. The participants can send this form back to the investigators to know whether they could be considered as subjects. Those individuals will be invited to an informational meeting (~ 1.5 hours), which will be held in a classroom at Virginia Tech.

At the informational meeting, the investigators will explain the purpose, the requirements and expectations, and the risks and benefits of the 5-week study. The investigators provide and review the Informed Consent Form with the participants and answer any questions or concerns they may have about the study. Individuals may either complete the full medical and schedule screening form at the meeting or turn in within two weeks of this meeting. Subjects will be selected from those who volunteer and qualify for the study.

A randomized, cross-over design will be used with two 14 d intervention periods with a 14 day wash out between treatments. Once the informed consent forms and screening forms have been received, the participants fitting our criteria will be randomly assigned into the raisin (R) supplemented or the placebo (PL) group for the initial part of the study. Investigators will ask the participants to discontinue use of any current vitamin and mineral supplements for 2 weeks prior to and until the end of the study. Subjects will be counseled on following a low-flavonoid diet (O'Byrne et al 2002, described below) for 2 weeks before the intervention. Baseline measurements will include body weight, height, waist and hip circumference, and acute metabolic and endothelial response to an acute high fat meal challenge. For the next 7 d all subjects will continue to consume a controlled, low flavonoid diet but add three snacks per day of the intervention or placebo. Half of the subjects will consume 90 g (270 kcal) raisins (as two servings per day of 45g each) while other half consume isoenergetic carbohydrate snack (jelly candy) each day. During the next 7 d, supplementation will continue but subjects will be fed all their food to insure careful compliance with the low flavonoid content (two meals at the laboratory facility and take away lunch). One raisin serving will be incorporated into each of the two meals consumed at our facility during the week of controlled feeding. All subjects will repeat the acute meal challenge test on d 7 of the controlled feeding with snack. Following a 14 d wash out period, the subjects will cross over to the other treatment for a second 14 d intervention period. A final acute meal challenge test will be performed after 14 d of this period. Blood samples will be collected on each day of the meal test at baseline (fasted), 120, 180, and 240 min after the meal. All venous blood samples ($\sim 40 \text{ ml}$) will be drawn by a certified medical laboratory technician.

All blood samples will be measured for serum metabolites and hormones (glucose, FFA, insulin, triglyceride), markers of oxidative stress (isoprostanes), markers of inflammation (CRP,

IL-6) while the fasted samples will also be measured for oxygen radical absorbance capacity (ORAC). Endothelial function (flow-mediated vasodilation, FMV) will be performed at baseline in the fasted state and at 3 h after each test meal.

Procedures

The low flavonoid diet will follow procedures as described by O'Byrne et al (2002). Subjects will be asked to avoid alcohol, tea, grape products, fruit juice, citrus, berries, onions, apples, and broccoli. For the controlled feeding period of the study, subjects will be fed two meals per day in the research facility and be given a prepared lunch and snacks. [Controlled feeding will be coordinated by B. Davy Ph.D, R.D.] The menu will be a 3 day rotation containing low flavonoids and approximately 30 kcal/kg, 55% carbohydrate, 30% fat, and 15% protein. Subjects will be asked to consume no other foods without permission during the two controlled feeding periods (including tea and other antioxidant containing beverages). The intervention snacks (raisins or jelly candy) will be provided at the meal and consumption will be verified by the experimenters. The high fat challenge meal will mimic that used by Esposito et al (2002): 760 kcal, 59% fat, 12% protein, and 28% carbohydrate (two sausages, six bread slices, cheese, butter, and olive oil) with the addition of either 90 g raisins or isocaloric jelly candy.

Blood samples will be taken via venipuncture, allowed to clot, centrifuged to separate serum, and frozen for later analysis. All blood venipunctures will be performed by a certified medical technician. Biochemical analyses will be performed on all measures in duplicate using commercially available kits:

Endothelial function

Flow-mediated vasodilatation will be measured as an indirect assessment of endothelial function. [performed by K. Davy. Ph.D. He has performed this procedure for previous studies] The change in brachial artery diameter, expressed as the percent change from baseline, will be imaged using a 7.5 MHz high resolution linear array transducer and ultrasound system (HP Sonos 5500, Phillips Medical) as previously described (Celermajer1994). Longitudinal B-mode images of the brachial artery will obtained 3-5 cm proximal to the antecubital fossa. Arterial diameter will be measured from the near to far arterial wall interface along a line perpendicular to the long axis of the artery. Temporary occlusion of the forearm circulation distal to the brachial artery will be performed by inflating a cuff placed around the forearm to supra-systolic pressure (10 min) as previously described (Celermajer1994). Images will be digitized at 30 Hz (Data Translations, Boston, MA) to computer for later analysis using commercially available software (Information Integrity, Boston, MA).

Freedom to Withdraw

During the initial information session the participants will be informed of their rights as study participants. Specifically, they will be told that they can withdraw from this study at any time and they will be free to not answer any questions or to not participate in any procedure included in this study.

Risks and Benefits

The interventions and procedures use in this study are considered of low risk to participants. Blood draws have a minimal risk. Occasionally, a bruise may result from blood

collection procedures with no known detrimental effects to the health or well being of the participant. To minimize bruising, a certified medical laboratory technician will draw all blood samples. Additionally, participants will be allowed to sit or recline in the most comfortable position for themselves during blood draws. Participants may rest for as long as needed after blood draws and will be provided with breakfast foods and beverages. Two attempts to draw blood (or two needle sticks) will be allowed. If a second attempt is unsuccessful, no further tries for blood collection will be performed. All personnel involved in blood draws and blood handling will have undergone training for Blood Borne Pathogen Exposure Control administered by the Environmental Health and Safety Services of the Occupational Health Lab Safety Division at Virginia Tech. Universal precautions will be taken by research personnel during handling of all blood samples. Standard operating procedures set by Virginia Tech's governing body will be executed in the event that blood exposure occurs.

There will be some pressure discomfort during the vascular function test when the cuff is inflated. This will be similar to that experienced with blood pressure measurement. There is no sensation with the ultrasound measurement.

Participants will be provided with any of their own data that they choose.

Confidentiality/Anonymity

Due to the inability to assure anonymity, confidentiality of the identity of each participant will be preserved. A code will be assigned to each individual participating in the study. All questionnaires, data collection sheets, data analysis sheets, blood collection and storage containers will be identified by code numbers. A master list of participants' code numbers will be kept in a locked filing cabinet separate from completed data, which will also be maintained in a locked filing cabinet. Only investigators involved in this study or future students of the principal investigator (faculty member) will be allowed access to any data.

Compensation

Subjects will be paid \$10 for completion of the first 2 week intervention, \$20 each for the pre and post testing in the first intervention period, \$50 for completion of the second 2 week intervention and \$200 for completion of the pre and post testing during the second intervention period for a total of \$300 for completion of the study.

Informed Consent

Participants will be fully informed about this research, including risks and benefits involved, and will agree to participate in writing by signing an Informed Consent form (see below). Each participant will be provided with the consent form at least one week in advance of any participation in this study. Each participant will be allowed to ask questions concerning the procedures. After questions have been answered, the participant will be given ample time to review the Informed Consent Form. Participants will provide Informed Consent by signing the form.

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Biographical Sketch

Janet Walberg Rankin is a Professor in the Department of Human Nutrition, Foods, and Exercise and Interim Director of the Institute for Biomedical and Public Health Sciences. She has been on the faculty at Virginia Tech since 1982. She earned her B.S. in Zoology from Duke University in 1977 and her Ph.D. in Nutrition from the University of California at Davis in 1982. Her research is primarily in sports nutrition and weight control for athletes and obese individuals.

Selected Publications (graduate student advisee):*

Smith, M.R.*, **J. Walberg Rankin**, H. Stevens* (2003) Effect of muscle glycogen on performance of repeated sprints and mechanisms of fatigue. International Journal of Sport Nutrition and Exercise Metabolism 13(1):1-14, 2003.

Rankin, J.W. Weight loss and gain in athletes (2002) Current Sports Medicine Reports1(4):208-213.

Wojcik, J.R.*, **J. Walberg Rankin**, L.L. Smith, F.C. Gwazdauskas (2001) Comparison of carbohydrate and milk-based beverages on muscle damage and glycogen following exercise. International Journal of Sport Nutrition and Exercise Metabolism 11:400-414.

Rockwell, J.*, **J. Walberg Rankin**, B. Toderico (2001) Creatine supplementation affects muscle creatine during energy restriction. Medicine and Science in Sports and Exercise 33(1):621-68.

Brenner, M.*, **J Walberg Rankin**, D. Sebolt (2000) The effect of creatine supplementation during resistance training on female athletes. Journal Strength and Conditioning Research 14:207-213.

Walberg Rankin, J. (2000) Dietary carbohydrate and performance of brief, intense exercise. Gatorade Sports Science Exchange #79, 13(4).

Selected Grants:

"Value of whey protein concentrate on glutathione status, exercise performance, and immunity in men during energy balance and restriction", Role as PI, Co-investigators: M. Houston, K. Saker. \$39,526.00 Funded from June 15, 2002 to August 30, 2003.

"Effect of Milk Consumption on Acute Muscle Protein Synthesis and Degradation after Resistance Exercise and on Chronic Muscle Hypertrophy", PI, National Dairy Council, \$88,942, April 1, 2000- July 31, 2001.

"Can High Levels of Muscle Glycogen Reduce Muscle Damage and Inflammation caused by Repeated High Intensity Exercise?" PI, Gatorade Sports Science Institute, \$6208, July 1998- July 1999.

"Muscle Glycogen, Sarcoplasmic Reticulum Function, and Fatigue. With student co-investigator Michelle Smith, American College of Sports Medicine Foundation, Reebok grant for students, \$2500, July 1998- July 1999.

Abigail D. Turpyn

Undergraduate Education:

B.S., 2000, Mary Washington College, Fredericksburg, VA. Biological Sciences.

Graduate Research and Education:

- Fall 2002-Fall 2003 Master's candidate in "Nutrition for Sport and Chronic Disease" option in Department of Human Nutrition, Foods, and Exercise; advisor: Janet Rankin, Ph.D
- Fall 2003 converted to Doctoral program (same option as above)
- Cumulative QCA 4.0 for 80 credit hours
- Research study, Fall 2003: investigating the effects of Atkins vs. Low-Fat diet on markers of inflammation and oxidative stress in overweight women, Study involved recruitment of subjects, diet education, blood draw and measurements, biochemical analysis of blood and urine samples.
- Research experience, Spring 2003: Research study assistant – whey supplementation study by Max Shute, 2003. VT, Involved separation of white blood cells from blood collection samples.
- Graduate Teaching Assistant, 2002 – present, Virginia Polytechnic Institute & State University, Blacksburg, VA. Department of Human Nutrition, Foods and Exercise.

- Instructor, Summer 2003, Virginia Polytechnic Institute & State University, Blacksburg, VA. Department of Human Nutrition, Foods, and Exercise.

Work Experience

Personal Trainer, Summer 2003, Virginia Polytechnic Institute & State University, Blacksburg, VA. Department of Recreational Sports.

Personal Trainer, 2001 – Present, Gold's Gym Aquia, Stafford, VA.

Kevin Davy, Ph.D.

Kevin Davy is an Associate Professor in the Department of Human Nutrition, Foods, and Exercise at Virginia Tech since Fall 2003. He was previously at the University of Mississippi Medical Center (2002-2003), and Colorado State University (1997-2002). He earned his doctorate in Exercise Physiology from Virginia Tech in 1992 and was a postdoctoral fellow/Instructor at University of Colorado from 1992-1997. Dr. Davy's research focuses on cardiovascular and sympathetic nervous system function in obese individuals and their relationship to body fat.

Selected Publications

- Davy KP**, T Horton, BM Davy, D Bessessen, JO Hill. Regulation of macronutrient balance in healthy young and older men. *International Journal of Obesity*, 25: 1497-1502, 2001.
- Beske SD, GE Alvarez, TP Ballard, **KP Davy**. Gender difference in cardiovascular baroreflex gain in humans. *Journal of Applied Physiology*, 91: 2088-2092, 2001.
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- Ho RC, **KP Davy**, BM Davy, CL Melby. Whole body insulin sensitivity, LDL particle size and oxidized LDL in overweight, nondiabetic men. *Metabolism*, 51(11): 1478-83.

Current Research Support

RO1 HL62283 Davy (PI)
NIH/NHLBI

2/01/00-1/31/04

“Abdominal Fat and Autonomic-Circulatory Control in Humans”

The major goal of this award is to determine the influence of abdominal visceral fat on autonomic-circulatory control in humans.

Role: P.I.

KO2 HL67227 Davy (PI)

4/01/01-3/31/06

NIH/NHLBI

Visceral Fat and Autonomic-Circulatory Control in Humans

Role: P.I.

Brenda Davy, Ph.D., R.D.

Brenda Davy is an Assistant Professor in the Department of Human Nutrition, Foods, and Exercise at Virginia Tech since Fall 2003. She earned registration as a dietitian in 1989, a Master's in Exercise Physiology from Virginia Tech in 1992, and a doctorate in Nutrition at Colorado State in 1992. She was an Assistant Professor in the Division of Hypertension at the University of Mississippi Medical Center from 2002-2003. Her primary research focus is the effect of dietary intervention on hypertension.

Selected Publications

Van Pelt RE, Jones PP, Davy KP, DeSouza CA, Tanaka H, **Davy BM**, and Seals DR. Regular Exercise and the Age-Related Decline in Resting Metabolic Rate in Women. *J Clin Endocrin and Metab.* 82: 3208-3212, 1997.

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Melby CL, Osterberg KL, Resch A, **Davy B**, Johnson S and Davy K. Effect of Carbohydrate Ingestion During Exercise on Post-exercise Substrate Oxidation and Energy Intake. *Int J Sports Nutr Exer Metab*, 12:294-309, 2002.

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Davy BM and Melby CL. A Review of the Literature: The Effect of Fiber-Rich Carbohydrates on Features of Syndrome X, *J Am Diet Assoc*, 103(1): 86-96, 2003.

Yu Y, Perret J, **Davy B**, Wilson J, Melby C. Antioxidant Properties of Cereal Products. *J Food Science*, accepted.

Davy B, Harrell K, Stewart J, King D. Body Weight Status, Dietary Habits and Physical Activity in Middle School-Aged Children in Rural Mississippi. Southern Med J, accepted.

Current Grants

R01 HL62283 (K. Davy)	2/1/00-1/31/04	50%
NIH/NHLBI	\$1,113,000	
Role: Co-Investigator		
Abdominal Fat and Autonomic-Circulatory Control in Humans		

Appendix G

Informed Consent for Participation of Investigative Projects

Virginia Polytechnic Institute and State University
Informed Consent for Participants of Investigative Projects

Title of Project: Effect of Diet on Oxidative Stress, Blood Vessel Function, and Inflammation in Obesity.

Investigators: Janet W. Rankin, Ph.D (PI), Abigail D. Turpyn, B.S., Mary Whitlock B.S. , Brenda Davy, Ph.D., R.D, Kevin Davy, Ph.D, Sean O'Keefe, Ph.D.

I. Purpose: The purpose of this study is to examine the effects of raisin or jelly candy consumption on levels of oxidative stress and inflammation in overweight/obese people. Various processes are known to increase the amount of chemicals that cause oxidation of chemicals in the body (e.g. smoking, strenuous exercise in unfit people, high fat and calorie meals). Oxidative stress and inflammation can be estimated by measuring several factors in the blood. Some research suggests that oxidative stress and inflammation may be linked to increased risk of heart disease, cancer and other chronic diseases. Overweight people usually have higher oxidative stress markers compared to lower weight individuals. Some research shows that changes in diet may reduce the oxidative stress levels in these people. We will be specifically testing the effect of two foods on measures of oxidative stress, inflammation, and blood vessel function in overweight individuals.

II. Procedures: Prior to being included in this research study, you will complete a Screening Questionnaire that will help determine if there are reasons why you should not participate in this study. These will include evidence of various health problems but also issues such as schedule and availability. If you meet the initial qualifications for the study criteria will be invited to attend an informational session held in a classroom on the Virginia Tech campus. During this session, the dietary regimen that the investigators will expect you to follow will be explained in further detail. Handouts that explain the requirements of the assigned dietary protocol, and that include lists of acceptable and non-acceptable foods will be provided during this time. If you are still interested, we will weigh you and measure the circumference of your abdomen. Experimenters will notify those subjects who qualify for the study within two weeks. If you qualify and you volunteer to participate, you will be assigned to undergo two treatments in a random order, separated by two weeks of eating what you like (total of 6 weeks). You will be asked to provide a blood sample for baseline measurement of some of the factors we are interested in. It is possible that this will eliminate you from the study at this point if we determine that your blood sample indicates an acute infection (specifically your C-reactive protein is higher than 20). If you continue passed this point, you will be asked to eat a low-antioxidant eating regimen [this means we will give you specific limits on several foods such as tea, some fruits and vegetables] for the six weeks of the study (4 as intervention weeks and 2 weeks between). Each two week intervention period will involve one week eating on your own (keeping food records) and one week when all your food will be provided by the experimenters. The latter will involve coming to Wallace Hall for two meals a day and being given one meal and snacks to eat away from the facility. This will be an ordinary diet except that it will be low in fruits and vegetables (1 each per day; although this is lower than recommended it is close to what the average American consumes). It is important that you do not consume any other foods from those provided during this week. And, it is important that you maintain your weight within 2 pounds. *This is not a weight loss diet.*

If you are selected to be in Group 1, you will be asked to consume 3 oz (about half a cup) of raisins each day for two weeks while when you do the other two week intervention you will consume the same number of calories (~270 kcal) as jelly candy (e.g. gummy bears). Two weeks of eating on your own will separate the two intervention periods. It is important that you consume all of the raisins or jelly candy each day of the intervention periods.

There will be tests of your physiological and metabolic response to a high-fat, high calorie meal for each of the two intervention periods (one at the beginning, and one at the end after two weeks of consuming the raisins or the candy). You will arrive after an overnight fast. We will take a blood sample from you. We will provide you with a meal consisting of about 800kcal (similar or equivalent to two sausages, six bread slices, cheese, butter) plus either the raisins or the jelly candy. More blood samples will be taken at 2, 3, and 4 h after the meal.

To summarize, if you are selected for this study, you understand that your participation will require approximately 4-hours ($\pm$ 30 minutes) of your time at each of four testing times. You also understand that you will be given ample opportunity to complete all procedures in an unhurried manner. You will be expected to discontinue the use of any vitamin or mineral supplements for the entire duration of this study. If at anytime during the study there are any changes to your present health or medical status, or you experience any unusual symptoms, you understand you need to inform the investigator immediately.

III. Risks: There is minimal risk involved in blood draws. A bruise may result from blood collection procedures with no known detrimental effects to your health or well-being. You understand that to avoid or minimize bruising, a certified medical laboratory technician will draw your blood. Additionally, you will be allowed to sit or recline in the most comfortable position for yourself during each blood draw. Two attempts to draw your blood (or two needle sticks) will be allowed. If a second attempt is unsuccessful, no further tries for collection of your blood will be performed. You understand that all personnel involved in drawing and handling blood have undergone training for Blood Borne Pathogen Exposure Control administered by the Environmental Health and Safety Services of the Occupational Health Lab Safety Division at Virginia Tech. You understand that precautions will be taken by research personnel during handling of your blood samples but that the standard operating procedures set by Virginia Tech's governing body will be executed in the event that blood or urine exposure occurs and includes HIV and hepatitis testing of your blood.

IV. Benefits of this Project: You will be provided, if desired, with the results of the study including information on endothelial function, oxidative stress, and inflammation status. At completion of this study, you may contact the investigator at a later date for a summary of the overall research results. You will be referred to an appropriate health care professional, if necessary, based on your individual results. Any and all costs related to such referral will be borne by you and not by Virginia Tech. The general public will benefit from your participation in this research as new understandings of links among dietary practices and chronic disease identified from this study.

V. Extent of Anonymity and Confidentiality: Due to the inability to assure anonymity, you understand that confidentiality of your results will be preserved. You understand that this means that all of your answers to questions that you are asked, measurements and laboratory values will be kept confidential. A code number will be assigned to you. All

questionnaires, data collection sheets, data analysis sheets, blood and storage containers will be identified by code number only and not by your name. You understand that a master list of participants' code numbers will be kept in a locked filing cabinet separate from completed data, which will also be maintained in a locked filing cabinet. You further understand that only the investigators of this study will be allowed access to any data.

VI. Compensation: You will be compensated for participation in this research project. Specifically, you will be paid \$10 for completion of the first 2 week intervention [or if you are asked to withdraw after the baseline blood draw due to evidence of acute infection], \$20 each for the pre and post testing in the first intervention period, \$50 for completion of the second 2 week intervention and \$200 for completion of the pre and post testing during the second intervention period for a total of \$300 for completion of the study.

VII. Freedom to Withdraw: You can withdraw from this study at any time. You are free to not answer any questions or to not participate in any procedure included in this study. You understand that there may be circumstances under which the investigator may determine that you should not continue to participate in this project. This could include evidence of health risk, weight loss, or non-compliance to procedures.

VIII. Emergency Procedure: If a minor emergency arises during your participation in this study, you will discontinue your participation and seek care from your personal physician. If a major emergency arises during your participation in this study, emergency personnel will be called (911), and they will care for you.

IX. Approval of Research: This research project has been approved, as required, by the Institutional Review Board for Research Involving Human Subjects at Virginia Polytechnic Institute and State University and by the Department of Human Nutrition, Foods and Exercise.

X. Subject's Responsibilities: You voluntarily agree to participate in this study. You have the following responsibilities:

- (1) Follow the dietary recommendations established by the investigators
- (2) Eat only the foods provided on the controlled feeding weeks
- (3) Maintain your weight within 2 pounds through the study.
- (4) Consume no vitamins or mineral supplements during the course of the study
- (4) Arrive at the laboratory on the scheduled days after having fasted overnight for a minimum of 12 hours;
- (5) Consume all of the dietary treatment (raisins or jelly candy) each day of the intervention
- (6) Allow the measurements to be conducted (weight, abdominal circumference, blood draws)

XI. Subject's Permission: You have read and understand the Informed Consent and conditions of this project. You have had all of your questions answered. You hereby acknowledge the above and give your voluntary consent for participation in this project. If you participate, you may withdraw at any time without penalty. You agree to abide by the rules of this project.

Participant's Signature

Date

Investigator's Signature

Date

Should you have any questions about this research or its conduct, you may contact:

Janet W. Rankin, Principal Investigator
(540) 231-6355

OR

Dr. David M. Moore, IRB Chair
(540) 231-4991

Appendix H
Instructions for Subjects

INSTRUCTIONS FOR FILLING OUT A FOOD RECORD

Please follow the directions for filling out your diet record carefully. The more accurate your record, the more complete your dietary analysis and recommendations will be. You will be completing a 3-day food record. Two days should be weekdays, one a weekend. Please record your name, day of the week and the date on which you completed your record on the top of each food record form.

Time: List the time you consumed the food or beverage

Food/Beverage: Write the name of the food or beverage

Amount: List the amount eaten or drunk in ounces (oz), teaspoons (tsp), tablespoons (tbsp), cups (c), in fractions of the aforementioned units, or in units such as “1 slice of whole wheat bread,” “1 raw orange,” etc (See Sample Food Record)

Description/Preparation: Describe methods used to prepare the food or beverage. Examples: baked, steamed, canned, fried, fresh, raw, frozen, with sauce, etc. Include brand names or restaurant names.

REMEMBER! Did you record all snacks you ate each day?

Did you record all beverages you drank? Such as: coffee, tea, water, milk, soda, juice, alcohol, etc.

Did you record any cream, milk or sugar added to your coffee or tea?

Did you record any lettuce, tomato, onion, etc. added to any sandwiches?

Did you record all condiments used? Such as: mayo, mustard, ketchup, salsa, sour cream, salad dressing.

Did you add butter or margarine to your food? Is it recorded?

Did you describe how each food was prepared (e.g. baked, fried, broiled, steamed or boiled)?

Amount Estimation Tools: One teaspoon = size of thumb above the joint

One cup = size of fist or walkman

One ounce cheese = size of thumb

3 oz meat = size of deck of cards

½ cup = size of tennis ball

TIPS:

- 1) Eat normally, don't alter food choices just because you have to record them
- 2) Finally, don't forget that your diet analysis is only as good as your record keeping!
- 3) Be honest!

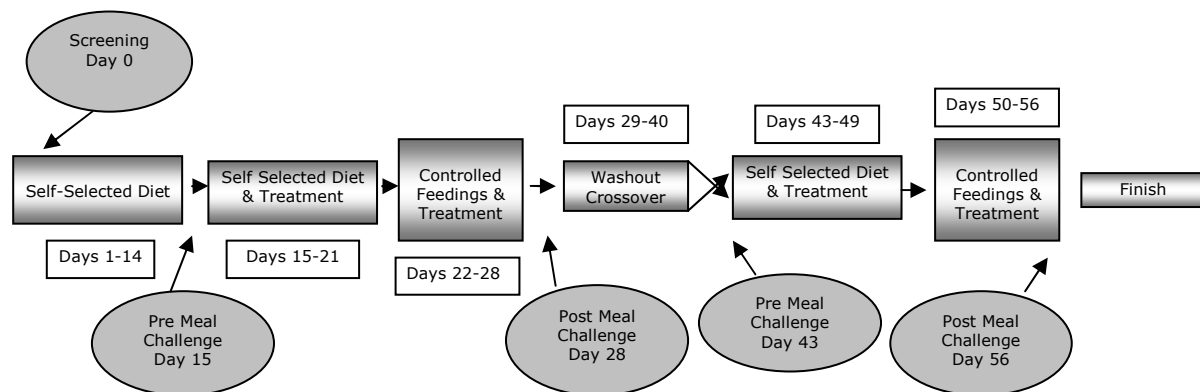
FOOD INTAKE RECORD

[illegible]

Description of Study:

It is an 8 week study (May-June) that involves following a low-flavonoid diet (low-antioxidant) for 8 weeks. The first 2 weeks you are eating on your own. We then assign you to 1 of 2 groups: fruit intervention or carbohydrate equivalent for 2 weeks of diet intervention. The second week of intervention involves eating all of your meals in our lab (VT campus) - take away lunch. Then 2 weeks of eating on your own again. The second part of the study is exactly the same but you will cross-over to the other treatment that you didn't receive before (fruit or carb. equiv.).

One very important scheduling factor that needs to be considered by all participants is the time commitment for the days of the blood draws. There will be 4 separate days of blood draws (and urine collections). Those days will require a 4 hour time commitment from 8AM until 12AM. During that time participants will be asked to provide one fasting first morning urine sample and multiple blood draws. Baseline blood draws will be followed by a meal challenge (standard fast food meal). After the meal blood draws will be taken at 2, 3, and 4 hours. After the final blood draw the participants will be free to leave. Please keep in mind that this will only occur on 4 separate days spread over the 8 week period. All blood draws will be taken by a trained phlebotomist.



Low-Flavonoid Diet:

The purpose of this diet is to eliminate any foods that might have a similar affect as raisins on some important measures in this study. In order to see the maximum benefit of the raisin supplementation we ask that you refrain from eating certain foods that may have this effect.

We understand that some of these foods may be ones that you are unwilling to part with. We will do our best to help you find a temporary substitute for the duration of the study.

Foods/Beverages to avoid are:

Fruits:

Berries: Blueberries, blackberries, strawberries, raspberries, cranberries
(any berries!)

Apples

Grapes

Plums

Citrus Fruits: Oranges, Grapefruit, Clementine, Tangerines, Lemon/Lime

Fruit Jams/Preserves

Vegetables:

Onions

Broccoli

Beverages:

Fruit Juices

Wine

Alcoholic beverages

Teas: Black, Green, Herbal, and Fruit

Grains:

Wheat Germ

Fortified Cereals

Fortified Cereal Bars

Fortified Granola bars

Power/Energy bars

Diet Supplements: SlimFast, Carnation Instant Breakfast/Bars, Atkins

products, etc.

Mixed Foods:

Any dishes containing the items above

Any foods with raisins (RaisinBran cereal, bars, breads, etc.)

If you have any questions about a food, any doubt about whether not you should eat it, please contact us **before** you consume the food!!!

Instructions for Screening Blood Draw: (Via email)

Concerning the lab day/blood draws, just a couple of quick things:

Please do not eat or drink anything after 9PM _____ night or before your blood draw in the morning (water's okay).

The blood draws will occur in Wallace Hall, room 231 on the main floor of the building. I believe that it is called the bone lab. The doors will be open and you won't be able to miss us. We will be sticking as close to the schedule as possible, with people coming and going it may get a little hectic, so please be patient :) The whole procedure shouldn't take too long. Keep in mind that we will be doing the height, weight, body measure of waist circumference at this time. So, it would be a good idea to wear comfortable, light, loose clothing if possible, and wear the same thing (or close to it) at each weigh-in (at each blood draw). The urine sample also should be quick, as long as you are hydrated going in (hydration is important!). Other than that, we look forward to seeing you!

Remember: We need your first morning urine!!!!

Your lab time starts at _____AM.

Instructions for Controlled Feeding:

Dos:

- Try to be on time – 7AM breakfast and 6PM dinner
- Please try to eat everything that is given to you – we don't want you to be hungry!
- If you are hungry let us know so we can adjust your diet
- Please bring any food that is not eaten back to the lab – don't throw anything away
- It's alright to save snacks for later in the day – you do not have to eat all of them for lunch
- Please voice your concerns and ask questions!

Don'ts:

- Do not wash lunch Tupperware – bring it back to the lab as is
- Do not consume any other foods outside of what we give you
- Do not consume any caloric drinks: Sodas, juices, milk, alcohol, etc.

Beverages that are okay:

- Diet sodas
- Sparkling waters
- Crystal lite
- Water
- Coffee w/non-caloric sweetener and a small (tsp) amount of creamer
- Other diet or non-caloric beverages not listed (no tea of any kind)

One last reminder:

Please consume only the foods that we provide during the controlled meals week. If at any time you are hungry and have eaten all of the items that we have provided for you, please contact us ASAP. We have a short list of acceptable “emergency” foods that can help you through until we adjust your diet.

Instructions for Treatment Consumption:

You will be given 7 days worth of treatment, each “dose” is in a labeled baggie: 1A, 1B....7A, 7B. You will be consuming 2 bags per day; eat “A” with breakfast and “B” with dinner.

IMPORTANT: Consume ALL of the treatment as directed.

If you receive the raisin treatment, please store raisins in the refrigerator and out of direct sunlight. If you receive the placebo, jelly candies, please store in a cool dry place. If you share a home with others, please explain the purpose of the treatment to avoid accidental consumption of the treatment.

Thank you

Appendix I

Meal Challenge Composition and Controlled Feeding Menu Example

Meal Challenge Composition:

Breakfast	Calories	Total Fat (g)	% fat	Carbohydrate (g)	% Carbohydrate	Protein (g)	% Protein	Fiber (g)	Vitamin C (mg)	Vitamin A (IU)
<i>Meal</i>	770	53		40		32		1	0	650
<i>Raisins (45g)</i>	134.5	0.2		35.6		1.45		1.7	1	0
<i>Placebo (35g)</i>	130.1	0		39		0		0	0	0
<i>Total w/Raisin</i>	904.5	53.2	52.9	75.6	33.4	33.5	14.8	2.7	1	650
<i>Total w/Placebo</i>	900.1	53	53.0	79	35.1	32.0	14.2	1.0	0	650

Fast food meals were provided on all 4 mornings of the test days: pre and post intervention on either side of the cross-over.

2500 kcal Day: Sample Menu during Controlled Feeding Period

Meal	Food
Breakfast	1.5 scrambled egg
	1 biscuit (60 grams)
	226.8g canned pears
	21.26g jelly
	45g raisins
Snack	4 peanut butter crackers
Lunch	357g chicken noodle soup
	8 saltine crackers
	1.5 grilled cheese sandwich: 3 slices white bread (50g), 1 slice American cheese (24.26g)
Dinner	cheese burger: 5 oz ground beef patty (70% lean), 1.5 (64.5g) hamburger bun, 24.26g American cheese slice, 9.17g mayonnaise, 23.44g yellow mustard, 24g iceberg lettuce
	45g raisins
Snack	56g pretzels
	2 puddings (116g each)

3-day rotating menus were utilized during the 7 d controlled feeding period. Diets were targeted to meet 1700, 2000, 2500, 3000, 3500 kcal/day requirements for weight maintenance. All menus were analyzed for nutrient composition prior to the commencement of the investigation (Nutrition Data System for Research (NDS-R version 5.0_35), Nutrition Coordinating Center, University of Minnesota, Minneapolis, MS)

Appendix J

Subject Data Sheets

Subject # _____ Checksheet

_____ Blood Draw

_____ Fasted Urine Sample

_____ Anthropometrics

_____ kgs

_____ meters

_____ waist

_____ hip

_____ 4d food record?

Questions on food record?

Questionnaire

- 1) Have you noticed any recent changes in health status? If so, explain....
- 2) How do you feel today?
- 3) What medications have you taken in the past 24 hours?

Date:_____

Subject # _____ Check sheet

Meal Challenge Day (circle number) 1 2 3 4

_____ Fasted Urine Sample

_____ Anthropometrics

_____ kgs = lbs_____

Supine Time:_____

Baseline Blood Draw (BD) Time:_____ Completed_____

Meal Challenge + Treatment Start Time:_____ Finish Time:_____

Supine Time:_____

2 Hour BD: Time:_____ Completed_____

Supine Time:_____

3 Hour BD: Time:_____ Completed_____

Supine Time:_____

4 Hour BD: Time:_____ Completed_____

Time completed:_____

Given Treatment:_____
(Days 1 and 3 only)

Date: _____

Subject # _____ Check sheet

Controlled Feeding Day: 1 2 3 4 5 6 7

_____ Anthropometrics

_____ kgs

Infection/Illness: NO YES

If YES please

describe: _____

Diet Kcal: _____

Waste:

Food	Amount (gms)	Amount not eaten

Treatment: Breakfast: _____

Dinner: _____

Appendix K

Exit Survey

Nutrition Intervention Study Exit Survey
Return to Mail code 0430 ATTN: Mary Whitlock

Subject # _____

Congratulations! You have completed the study. Before you leave we would like you to answer a few questions. You cannot be penalized for your responses so please be COMPLETELY honest. We enjoyed your participation and hope you will consider other study opportunities that may come along from the HNFE Dept.

1. During the course of the study, did you eat any of the foods that you were instructed to avoid? (fruits, vegetables, wine, tea, etc.). If so, please indicate when you ate them, what items, and how much.

2. Did you consume 100% of the treatments you were assigned? (Jelly beans and raisins). If not, please indicate the amount of the treatment that was not taken and when.

3. During the controlled feeding periods, did you at any time eat or drink (excluding non-calorie drinks) anything outside of the food lab? If so, please indicate when you ate them, what items, and how much.

4. On a scale of 1-10 (10 being extremely difficult), how difficult was it to follow a low-flavonoid (antioxidant) diet?

5. On a scale of 1-10 (10 being the most difficult), how difficult were the blood draw mornings? Explain.

6. Please provide any comments on how the study was run and any ways we could have been more helpful or done a better job.

7. Do you want any suggestions concerning your diet quality? Weight loss?

Appendix L

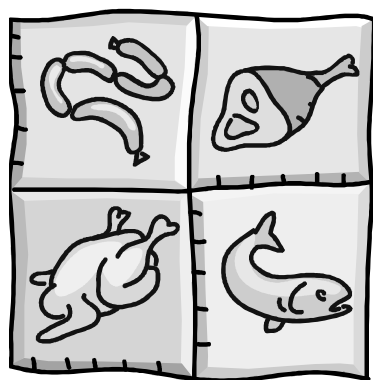
Recruitment Fliers and email

Participants Needed for a Diet Intervention Study

Sponsored by the Virginia Tech
Department of Human Nutrition, Foods, and Exercise

If you qualify for this study you will be placed on a supervised Diet intervention and learn about your:

- Body composition
- Heart Disease Risk
- Nutrition quality of your current diet



If you are:

- 18-50 years of age
- Overweight (>20lbs)
- Non-smoker
- Willing to participate in a 8 week study

You may qualify for this study

DON'T DELAY!! The study begins in May 2005. We will be screening for eligible participants for the next 4 weeks. Please contact us ASAP to see if you qualify!

For more information, please contact:

- Mary Whitlock, raisinstudy@hotmail.com

Earn \$300!

Earn \$300!

Earn

If you are:

- 18-50 years of age
- Overweight (>20lbs)
- Non-smoker
- Willing to participate in a 8 week study

You may qualify for this study

[illegible]

Recruiting email sent via VT Graduate School Listserv:

Researchers in the Department of Human Nutrition, Foods, and Exercise at Virginia Tech are recruiting healthy, nonsmoking overweight men and women to participate in a nutrition intervention study investigating the effects of fruit consumption on markers of cardiovascular disease risk. Potential subjects need to be between the ages of 18-55, overweight (at least 20 lbs), and sedentary. If you meet these criteria, you may qualify for this study. Study participants will be asked to maintain their weight throughout the study while following our dietary requirements. Benefits of participation include: Two weeks of planned meals provided at our facility, assessment of body composition, nutrition quality of your current diet, and some indicators of cardiovascular disease risk. Additionally, participants that qualify will also receive monetary compensation (\$300) for their time.

If you're interested, please contact Mary Whitlock or Abby Turpyn at raisinstudy@hotmail.com.

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